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ALBINISM, PARTIAL ALBINISM, AND VITILIGO

Albinism, partial albinism, and vitiligo are three clinically distinct conditions having in common a deficiency of melanin pigment. As such they present the physician with the same basic problems—cosmetically disfiguring lesions and extreme sensitivity to sunlight of the involved areas. None of the diseases is itself an immediate threat to life, but the social disabilities, especially among dark-skinned individuals, are of great magnitude. Each disorder has been well known for over a century, and many detailed case reports are on record. Vitiligo, being by far the most common of the three, has received the greatest amount of attention. Albinism and partial albinism, because of their obvious characteristics, have received their share of study, especially from geneticists. To our knowledge, however, there has not been an attempt to correlate the available information on the three conditions. This is the purpose of the present report.

Albinism and vitiligo were dealt with extensively elsewhere,[9, 11] and new material will be confined to the first published example of a nevus from a human albino. Partial albinism is less well known and will be described in more detail. A new family pedigree—only the seventh to be reported in the United States—will be presented, and for the first time a histological study will be reported. Finally, the pertinent information will be summarized in table form for convenient reference.

ALBINISM

Clinical manifestations. Albinism is the best known and most adequately studied of the various disorders of pigmentation. In its classical form it is unmistakable, because from birth the individual is completely devoid of melanin in all tissues where this pigment is normally present—skin, hair, and eyes. However, many albinos are capable of a very limited degree of pigment formation, particularly as they grow older. From the standpoint of

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color they may appear indistinguishable from a very blond Caucasian. These lightly pigmented persons often are referred to as incomplete albinos by geneticists and as partial albinos by ophthalmologists, the latter being consulted because of the patients' visual difficulties. The term 'partial albino' is not a good one since it is used by dermatologists and geneticists to refer to an entirely separate disorder which will be discussed below.

The most characteristic findings in albinism, aside from the obvious absence of skin and hair pigment, are in the eyes. The non-pigmented iris and retina give the pupil and iris a reddish appearance and, as might be expected, result in severe photophobia. The vessels of the choroid and retina are very prominent on fundoscopic examination. There may or may not be an associated hypoplasia of the macula.[9] A searching nystagmus is usually present associated with severely impaired visual acuity. Often it is this amblyopia which is the patient's major source of difficulty. "Ocular albinism" may occur as an isolated finding with otherwise normal pigmentation.

Other congenital anomalies may be associated with the pigment defect in albinism. They probably are an expression of multiple recessive traits resulting from a consanguinous mating rather than a product of the same genetic defect that caused the hypopigmentation.[9] Mental deficiency and short stature often have been described as associated findings.[8]

Rarely will any difficulty be encountered in identifying a true albino. The characteristics differentiating him from a person with hypopigmentation of another etiology are the following: (i) generalized lack of pigment; (ii) congenital onset with minimal change in pigmentation occurring throughout life; and (iii) ocular nystagmus with amblyopia and other congenital anomalies.

Incidence. On a world-wide basis the incidence of albinism is about one in 20,000 population.[9] In some areas, because of consanguinity, the incidence is much greater. There is no known difference in incidence between the sexes except that only males have the form of the disease confined to the eye.

Etiology. The etiology of albinism is generally assumed to be a genetically determined absence of the enzyme tyrosinase which is essential for the oxidation of tyrosine to dihydroxyphenylalanine and other intermediates in the pathway of melanin synthesis. This assumption is based upon the repeated failure to demonstrate tyrosinase activity upon *in vitro* incubation of albino skin and hair in suitable substrates. Unanimity of opinion is still lacking since it is known that under various circumstances a limited amount of pigment formation can be stimulated.[7, 10] The nystagmus and other ocular changes have not been satisfactorily explained.

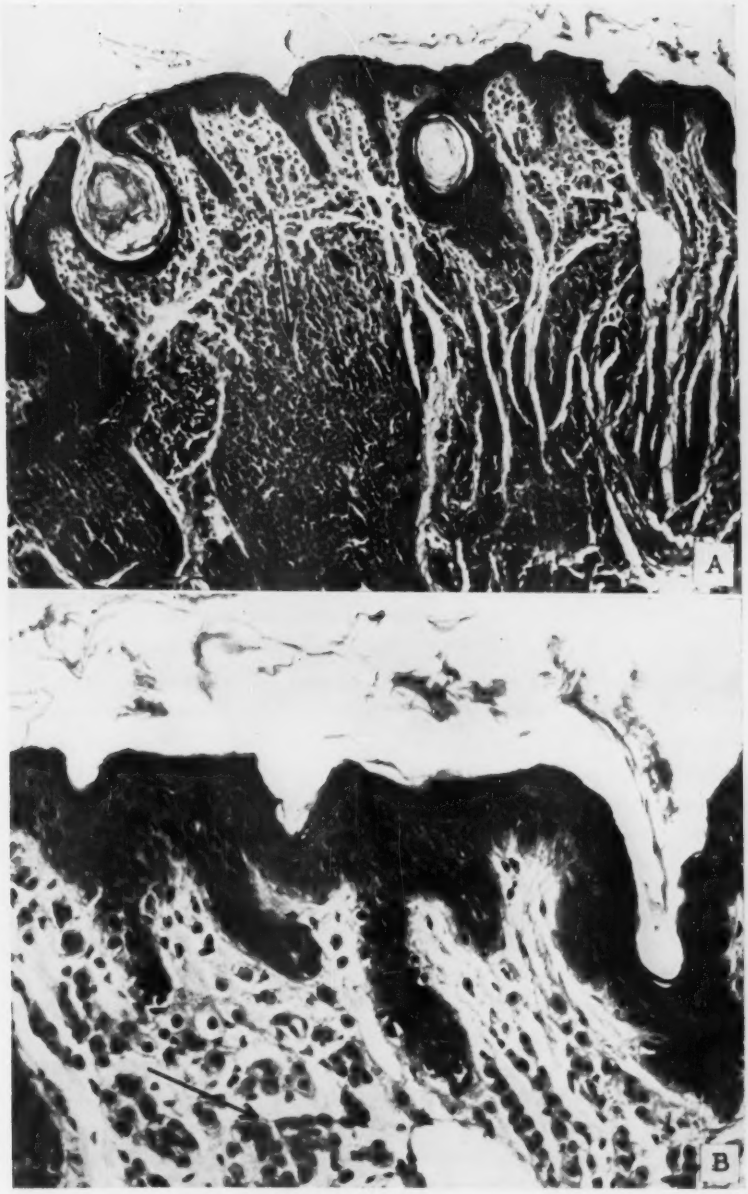


FIG. 1. Intradermal nevus from a total albino showing an accumulation of clear cells or melanocytes in the dermis. Melanin granules are not present. Stained with hematoxylin and eosin. (A) mag. x100. (B) mag. x265.

Histopathology. It has been shown beyond any reasonable doubt that the pigment forming cells, or melanocytes, are present in normal numbers in human albinism.[1, 2, 10] Nevi composed of non-pigmented melanocytes have been found to occur, although the example presented here (Fig. 1) is apparently the first to be published. A few instances of melanomas, made up of non-pigmented, anaplastic melanocytes have also been reported in albinos.[17] The histochemical techniques used to demonstrate melanin and melanocytes are of no use in histological studies of albinism. That is to say, darkening of the cytoplasm of the melanocyte by dopa or tyrosine, which requires the presence of active tyrosinase, does not occur; and the reduction of silver salts, which is performed by the melanin granules, also cannot take place.[6] Since this phenomenon is characteristic of all the disorders in the group it cannot be used as a diagnostic criterion.

Genetics. The mode of inheritance of total albanism has been studied carefully by numerous investigators. Almost invariably the defect has been inherited as an autosomal recessive.[8] By contrast, inheritance of ocular albinism is well documented as a sex-linked recessive. Female carriers of the trait for ocular albinism show characteristic pigmentation changes in the retina.[6]

Therapy. For practical purposes there is no therapy. Tinted eyeglasses and protection of the skin from excessive sunlight are the only convenient symptomatic measures.

Prognosis. The prognosis in patients with albinism depends essentially upon the nature of the associated defects. The absence of melanin *per se* is not incompatible with a normal life span, but survival is difficult in afflicted persons exposed to intense and prolonged sunlight, as in the tropics, because there is an increased incidence of skin cancer[18] in such individuals. With aging, some patients develop increased pigmentation, accompanied by improvement in visual acuity. This, plus the fact that they learn to accommodate to their handicap at an early age, means that often a satisfactory adjustment to environmental conditions can be made.

PARTIAL ALBINISM

Clinical manifestations. Partial albinism is entirely distinct from total albinism and is not to be confused with incomplete albinism. It also is known by many other names including piebaldism, white-spotting, white forelock, congenital vitiligo, albinoidism, congenital achromia, etc. Perhaps the term partial albinism should not be used because of possible ambiguity, but since it is the most familiar it will be adopted here.

This disorder is characterized by localized patches of hypopigmentation which are present from birth and do not change in size throughout life. The remainder of the body is of normal color. The most familiar form of the disease is that manifested by the white forelock. Usually the skin lesions are distributed over the ventral thorax, abdomen, extremities, brow, and scalp under the white forelock.[4] The lesions are bilateral and asymmetrical, vary greatly in size, and may contain islands of normally pigmented skin. There is no hyperpigmented border as seen in some patients with vitiligo, but there may be varying shades of melanin pigmentation in a single subject (Fig. 2). The eyes are not involved. Characteristically, other members of the patient's family have similar depigmented areas since the disorder is inherited as a dominant trait. The patients presented in this report showed remarkably similar lesions, but this is not always the case. In contrast with total albinism, there are no associated anomalies. The patients are asymptomatic unless there is much involvement of skin in the exposed areas with no protection against sunlight.

In summary, partial albinism can be differentiated from total albinism by the circumscribed nature of the lesions and by the absence of nystagmus or other constitutional abnormalities. Unlike vitiligo, the lesions of partial albinism are present at birth and do not change. The hyperpigmented border which is often seen in vitiligo is not present in partial albinism. Examination of other members of a patient's family may be helpful, but since vitiligo may appear as a dominant trait in a family, this may prove misleading.

Incidence. The true incidence of partial albinism has not been determined, but it is certainly more common than the approximately 25 cases recorded in the literature would indicate. One author believes the incidence to be about equal to that of total albinism.[18] Many individuals probably never seek medical attention because they are asymptomatic, and their lesions are insignificant.

Etiology. No satisfactory theory has been proposed to explain this disease. Many feel that it is analogous to white-spotting in the coats of lower mammals.

Histopathology. Although the disorder is clinically and genetically similar to white-spotting in lower mammals, the histological findings are notably different. In the white areas of spotted mice, rats, and guinea pigs, pigment cells identified as "clear cells" are totally lacking.[14, 16] Skin biopsies taken from a lesion of the patient presented below by contrast showed clear cells or melanocytes, normal in number and morphology[10] (Fig. 3). Hence the lesions of partial and total albinism are histologically indistinguishable.

FIGURE II

Father and daughter with partial albinism. Note distribution of lesions and their similarity on the backs of the two patients. Note also the hyperpigmented areas on the back.

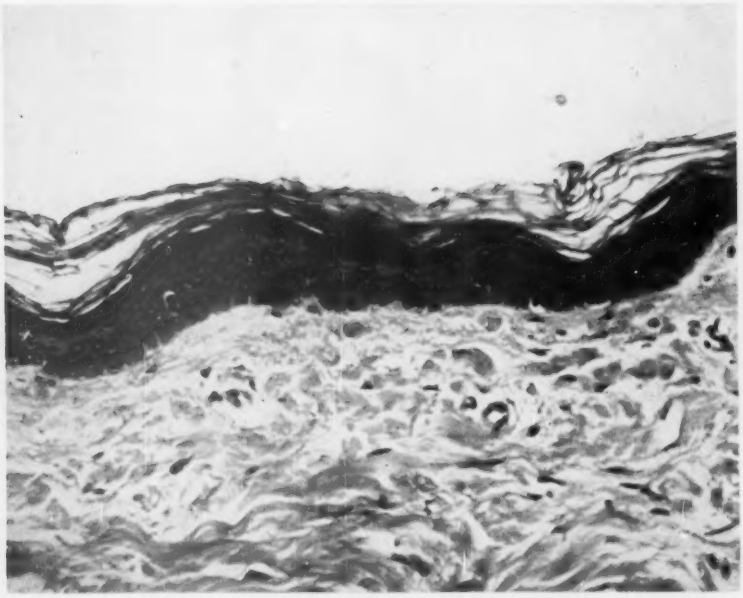
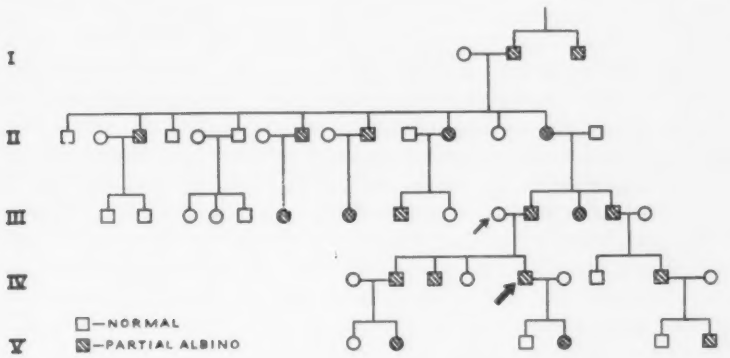


FIG. 3. Biopsy section of a lesion in partial albinism showing the presence of melanocytes, identified as clear cells, in the basal layer of the epidermis; mag. x265.

Genetics. Virtually the entire existing literature on this disorder is concerned with its mode of inheritance, which has been well worked out as an autosomal dominant. Thus it is to be expected that 50 per cent of individuals in an affected family will show the defect in some manner.

Prognosis. The lesions themselves are permanent and never change. There are no constitutional effects from the disease so that health and life expectancy are usually normal.

Case Report. R. A., a 38-year-old Caucasian male, was first seen together with his 5-year-old daughter, G. A., in the Dermatology Clinic of the Yale-



PEDIGREE OF "PARTIAL ALBINISM" IN A NEW ENGLAND FAMILY
(BIRTH ORDERS ARE NOT INDICATED)

FIG. 4. Pedigree of partial albinism demonstrating dominant pattern of inheritance. Arrows refer to informants, the heavy arrow denoting the father described in the report.

New Haven Medical Center in 1956. Both showed extensive areas of depigmented skin which had been present since birth without change in size or color. In addition, the girl had a triangular white blaze of hair. Both patients were asymptomatic and were concerned mainly with the cosmetic appearance of the defect. The skin in the affected areas burned on exposure to sunlight and had no capacity for tanning. The remainder of the body tanned normally. Further questioning of the father and his mother revealed other members of his family with the same disorder as indicated in the accompanying pedigree (Fig. 4).

Upon examination both patients were physically normal in every respect except for the characteristic skin lesions of similar distribution over the scalp, face, thorax, back, and extremities (Fig. 2). Some parts of the skin were hyperpigmented and contained macules that looked like *café au lait* spots.

VITILIGO

Clinical manifestations. Vitiligo is a common disease of considerable importance in the practice of medicine. It is highly variable in its manifestations and has aroused much curiosity among pigment researchers. The lesions may range in size from a single, small circumscribed area of depigmentation to "complete" vitiligo in which virtually the entire body is involved. Commonly there are multiple patches which may or may not be symmetrical or segmental in distribution. The exposed areas are usually involved, but the lesions may appear anywhere on the body surface. Depigmentation may be partial or complete. Hair may be gray (white) or uninvolved. Lesions often have a hyperpigmented border with rings of gradually increased pigmentation extending peripherally. Another characteristic is the perihalo nevus which is a pigmented nevus surrounded by an area of depigmentation. Vitiligo may begin at any age. It tends to come on early, with 50 per cent of the cases occurring before the age of 20 years in one study.[11] Onset of the disease may be associated with physical or emotional trauma. Some patients have co-existent organic or emotional illness. An increased incidence of vitiligo in patients with pernicious anemia, hyperthyroidism, and Addison's disease has been reported.[11] The disease has periods of quiescence or progression which are usually unpredictable. Lesions spread peripherally, and new ones may appear, too. The psychological implications, particularly in the darker races, are enormous.

Vitiligo may be distinguished clinically from albinism and partial albinism by the following points: (i) Vitiligo usually begins after birth; (ii) the lesions change in extent and severity; and (iii) hyperpigmented borders of depigmented areas and perihalo nevi are common in vitiligo. In the differential diagnosis, vitiligo must be distinguished from acquired hypopigmentation due to other causes such as trauma, syphilis, atopic dermatitis, etc.

Incidence. A number of studies have been undertaken to determine the incidence of vitiligo in various parts of the world, with estimates ranging from 0.14 to 3.2 per cent. It is possible that the disease is more prevalent among dark-skinned races, but this higher incidence may be more apparent than real. It is probably safe to state that the incidence of vitiligo on a world-wide basis is about one per cent.

Etiology. There is much evidence to suggest that vitiligo results from the presence in the skin of a yet unknown neurohormone that can lighten melanocytes.[11]

Histopathology. As in the previously discussed disorders of pigmentation, melanocytes are present in normal numbers in the lesions of vitiligo. In the hyperpigmented border which is often present they appear to occur

TABLE 1. FEATURES OF ALBINISM, PARTIAL ALBINISM, AND VITILIGO

	Albinism	*Partial albinism*	*Vitiligo*
Incidence	1:20,000	Unknown (rare)	1:100
Etiology	Deficiency of tyrosinase	Deficiency of tyrosinase	Excess amounts of neurohormone in skin
Extent of lesion	All tissues containing melanin	Discrete, circumscribed areas of skin and hair	Variable — usually discrete lesions of skin and hair
Sites commonly involved	Entire body, but eyes alone may be involved	Any area, particularly scalp	Exposed areas, esp. dorsum of hands, about body orifices and in body folds
Characteristic findings	Amblyopia and searching nystagmus	White forelock	Hyperpigmented border of lesion, perihalo nevus
Associated illnesses	Other congenital defects	None	Hyperthyroidism, pernicious anemia
Age of onset	Congenital	Congenital	Any age but usually by early adulthood
Clinical course	A minimal degree of pigment formation may occur with increasing age	Lesions do not change in relative size throughout life	Exacerbations and remissions characteristic; repigmentation may occur
Histopathology	Dopa-negative melanocytes present	Melanocytes present	Melanocytes present with little or no dopa activity
Heredity	Autosomal recessive; ocular form sex-linked	Autosomal dominant	Inconsistent — may show dominant pattern

with an increased population density and are strongly reactive upon incubation in solutions of dopa or tyrosine.[10] The melanocytes in the areas of depigmentation show reduced or absent tyrosinase activity.[8] The findings otherwise are non-specific. Inflammatory cells may be evident sub-epidermally but their significance is unknown.

Genetics. Vitiligo tends to occur in families and may show a pattern consistent with a dominant mode of inheritance. There obviously must be

modifying factors affecting the expression of the trait since the majority of patients have no relatives with the disease.[13] More detailed statistical study is necessary to clarify this point.

Prognosis. Vitiligo, like partial albinism, has no direct effect upon health or life expectancy. However, patients with this disease may be disabled for cosmetic reasons, and they may burn excessively when exposed to sunlight. So far as the lesions themselves are concerned, the course is variable with periods of progression and quiescence being common. Physical or emotional trauma may initiate an exacerbation. Repigmentation of some degree occurs in about 50 per cent of patients. This begins from the periphery of the lesion or from perifollicular foci in areas where the hairs are still pigmented.[28] Complete cure may result, but this is uncommon.

The accompanying table summarizes the important and distinguishing features of albinism, partial albinism, and vitiligo.

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THE ROLE OF ANTIDIURETIC HORMONE IN CORTICOTROPHIN RELEASE**

The possibility that the posterior lobe of the pituitary may provide the neuroglandular link for activation of the anterior lobe was entertained by Hensey and Markee as early as 1933 when they postulated that "the pathways from the hypothalamus must activate the posterior lobe of the hypophysis which in turn may exert an influence on the anterior lobe by hormonal transmission."[28] This relationship has become of more than theoretical interest since vascular links between the anterior and posterior pituitary glands have been demonstrated in the rat,[40] dog,[42] rabbit, cat, guinea pig,[17] and man.[34] The functional significance of these interlobar vessels has been demonstrated by their ability to maintain the integrity of a significant volume of anterior pituitary following infarction of the stalk of the anterior lobe in the rat, sheep, goat, baboon, rhesus monkey, and man.[10]

At the present time, evidence has been advanced to demonstrate a role of the neurohypophysis in the control of luteotrophic,[5] thyrotrophic,[28] somatotrophic,[18] and adrenocorticotrophic hormone release. The evidence that ACTH may be regulated by the neurohypophysis lies along three lines: (i) the administration of exogenous vasopressin results in ACTH discharge; (ii) ADH and ACTH are released or inhibited simultaneously; and (iii) the posterior pituitary is essential for the release of ACTH.

Stimulation of ACTH release by exogenous ADH. The repeatedly observed effect of exogenous vasopressin upon adrenal activation is the best documented of the arguments for its role in ACTH release. The effect of posterior lobe extracts upon the adrenal cortex was first noted by opotherapists. Baduel, as early as 1908, noted adrenal cortical proliferation resulting from administration of *succo de lobo posteriore ipofisurie*.[8] In the following year Delille published results of experiments often

* Interne, Pediatric Service, Grace-New Haven Community Hospital.

** The author acknowledges the help of Dr. Roger Guillemin and Dr. Alvin Brodish in the preparation of this paper. Fellowships were received from the following sources during tenures in their laboratories: Baylor University College of Medicine, Texas Medical Foundation, and USPHS Summer Fellowship. Portions of this paper were included in the thesis submitted to the Yale University School of Medicine in 1960 as partial requirement for the degree of doctor of medicine.

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said to be the first to demonstrate the corticotrophic effect of the pituitary gland. It is not generally appreciated that he attributed this activity to the posterior pituitary. He demonstrated that the administration of either whole extract or posterior pituitary extract doubled adrenal weight, caused hyperplasia of adrenal cortex, and produced pituitary basophilia[11] (confirmed by Martini *et al.* 1956.[58]

The investigations of the opotherapist lay fallow for more than two decades until their studies were confirmed by several investigators seeking to find in the pressor activity of the posterior lobe a possible etiology of vascular disease. Moehling and Osius in 1931 and Hantschmann in 1937 noted the hypertrophy of the zona fasciculata of the suprarenal cortex in response to vasopressor fractions of the pars neuralis.[35, 48] In 1935 Moszkowska noted the presence of corticotrophic activity of the pars neuralis when injected into normal rabbits. She attributed this to diffusion of colloid containing ACTH from the anterior lobe.[65]

In his attempts at ACTH purification Sayers noted in 1943 that posterior lobe contamination of ACTH produced an additive adrenal response in the normal animal. This effect was localized in the vasopressin fraction and was abolished by hypophysectomy of the recipient animal.[80] Parks some eight years later also remarked about this property of the posterior lobe extracts.[73]

The first intensive investigation of this activity of vasopressin was published by Eser and Sipahioglu who demonstrated an eosinopenia when this material was injected into normal rats. They demonstrated that this phenomenon was inhibited by hypophysectomy but not by unilateral adrenalectomy or adrenal demedullation. On the basis of the anatomical proximity of the pituitary lobes, they postulated that vasopressin may be one mediator of ACTH stimulation.[18] In the same year Nagareda and Gaunt noted similar adrenal activation while studying the apparent reciprocal nature of ADH and ACTH at the renal level.[69]

In the following year Stutinski and his collaborators demonstrated the posterior lobe activity in a number of commercial preparations and were able to show that the adrenal activating activity is parallel to the vasopressor activity, thus concluding that the property must be due to vasopressin.[91]

At the present time there are some 25 published experiments decribing the adrenal activation following injection of vasopressin in normal animals (Table 1). The indices of this activity have been varied: eosinopenia, anti-insulin effect, adrenal weight, adrenal histology changes, adrenal content of cholesterol and ascorbic acid, P^{32} uptake by the adrenal, plasma and urinary steroids, and plasma or pituitary ACTH. The preparations of vasopressin

TABLE 1. EFFECT OF VASOPRESSIN IN THE NORMAL ANIMAL* **

Vasopressin	*Dose U.*	*Animal*	*Index*
Pitressin	7.5/15 days (SC)	Rat	Adrenal weight, lipid depletion[53]
Pituitrin	0.28-0.5 (SC)†	Rat	Cytochemical changes in adrenal cortex[4, 91]
Pitressin	1.0-5.0 (IP)	Rat	Adrenal cholesterol depletion[89]
Pitressin	0.1-0.2 (IP)	Mouse	Anti-insulin effect[60]
PNH	0.83-1.66 (IP)		
Pitressin	0.7-1.25 (IV,SC,IP)	Rat	Adrenal ascorbic acid depletion[5, 8, 14, 15, 40, 44, 66, 91]
Crude VP	0.4 (SC)		
Purified VP	0.4 (SC)		
Vasopressin A,B,C,(?)	0.5 (SC)		
Pituitrin (DNZ)	0.4 (SC)		
LVP	0.3 (IP)		
Saline extract post. pituitary	—— (IP)	Guinea pig	Urinary neutral reducing lipid[90]
Pitressin	3.0 (IP)		
Highly purified	8.0 (IP)		
Highly purified LVP	0.13-2.12 (IC)	Dog	Plasma 17-OH corticoids[70]
Pitressin	2.0-5.5 (IV)	Human	Plasma 17-OH corticoids[58, 59, 92]
LVN—2	2.0 (IV)		
AVN—2	2.0 (IV)		
Synthetic LVP	2.0 (IV)		
Pitressin	0.2 (IP)	Rat	P^{32} uptake by adrenals[67]
Pitressin	2140/100 days (SC, IP)	Rabbit	Adrenal weight[63]
Tonephin		Rabbit	Adrenal weight[85]
Vasopressin (DNZ)	0.2-1.0% (SC)	Mouse	Cytochemical changes in adrenal cortex[46]
Post. Pit. extract		Rabbit	Adrenal weight[65]

TABLE 1. (Continued)

Vasopressin	*Dose U.*	*Animal*	*Index*
Infundin	0.005† (SC) 0.1-2x0.25 (SC)	Rat	Eosinopenia[18]
Postuitrin	0.01† (SC)		
Pitressin	0.08-0.1† (IP,IV) 0.2 (IP)	Rat	Adrenal ascorbic acid depletion, pituitary ACTH content (*in vitro* assay)[76]
Synthetic AVP	0.04-0.4† (IV)		
Pitressin	0.04† (IC) 0.08-0.2 (IC)%	Rat	Plasma ACTH activity (adrenal ascorbic acid assay)[8]
Synthetic LVP	0.2 (IC)%		
Highly purified AVP	0.002 (VNT) 0.02† (IV)	Dog	Adrenal effluent 17-OH corticoids[47]

* *Symbols:*

%—dose stated per 100 gm. body weight.
SC—subcutaneous injection.
IP—intraperitoneal injection.
IV—intravenous injection or infusion.
IM—intramuscular injection.
IC—intracarotid infusion or injection.
VNT—intraventricular injection.
†—ineffective.
local—direct application to intraocular transplant.
perf.—perfusion per minute.
VP—vasopressin.
LVP—lysine vasopressin.
AVP—arginine vasopressin.
(?)—preparation of vasopressin not stated.
LS—lymph sac of frog.

** *Preparations of Vasopressin:*

Pituitrin, Pitressin—trademarks of Parke-Davis.
Postipofisan—trademark of Richter.
AVN—2,3,5 arginine vasopressin purified by du Vigneaud.
LVN—2 lysine vasopressin purified by du Vigneaud.
PNH—Pars neuralis hormone prepared by Van Dyke.
Pituitrin(DNZ)—product of Dai-Nippon Zoki Institute.
Vasopressin(DNZ)—product of Dai-Nippon Zoki Institute.
Tonephin
Infundin
Postuitrin

have ranged from posterior lobe extracts to synthetic (and therefore presumably pure) arginine and lysine vasopressin. In addition it has been shown that inactivation of the pressor activity parallels inactivation of ACTH releasing activity.[9] Although we can conclude from these studies that vasopressin stimulates ACTH release, we must also discuss the specificity of this effect and its physiological significance.

Because of the possibility that vasopressin may act as a nonspecific stressor, preparations have been employed in which the ACTH response to various stresses has been inhibited or abolished (Table 2). The 23 published attempts to demonstrate a specificity of response have employed various preparations which include: the immature rat, the steroid block, the morphine-nembutal block, chloropromazine block, prolonged ether anesthesia, hypothalamic lesions, ocular pituitary transplants, decerebrate and ether anesethetized rats, neurohypophysectomy, and pituitary stalk section. In each of these experimental situations the preparation has shown an inability to respond to one or more stressing procedures, indicating some degree of isolation of the pituitary gland from its afferent stimuli. The demonstration that vasopressin is specifically active in these circumstances makes the conclusion that the material acts directly upon the pituitary seem justified. This conclusion is strengthened by the observation that synthetic lysine vasopressin has a corticotrophin releasing effect upon the pituitary *in vitro*.*[54] It is noteworthy that the systemic doses necessary for adrenal activation are in general the same as those used in the normal animal.

Nagureda and Gaunt were the first to question the physiological significance of the ACTH releasing effect of vasopressin when they studied the phenomenon in 1951. In their classic work they noted that the dose of pitressin necessary for adrenal activation was 20 to 80 times that reproducing physiological antidiuresis (0.005 units).[66] Shannon and later Lausen studied the rate of ADH liberation in dog and man and found that the physiological rate of secretion fell between 0.001 to 0.005 and 0.007 to 0.050 units respectively.[60, 65] It is readily appreciated (from Tables 1 and 2) that all of the doses administered systemically were in excess of the physiological range. This forms one of the serious objections to the ADH theory of pituitary stimulation.

Although this objection to the significance of ADH in activating the adrenal may be partially on the basis of esthetics, some attempts have been made to quantitate the threshold necessary to produce ACTH release.

* It is pertinent to call attention to the criticisms of *in vitro* studies of pituitary and adrenal stimulation. Those interested in this topic of methodology are referred to a recent review and defense of the *in vitro* technique by Guillemin and Schally.[24]

TABLE 2. THE EFFECT OF VASOPRESSIN IN ANIMALS BLOCKED TO STRESS*

Vasopressin	*Dose U.*	*Animal*	*Index*
	Immature		
Pitressin PNH	35/3days (IP) 25/3days (IP)	Rat	Adrenal hypertrophy lipid depletion[80]
Pitressin	1-5 (IP)	Rat	Adrenal cholesterol depletion[80]
Pitressin PNH	35 (IP) 25 (IP)	Rat	Thymus involution body weight[80]
	Steroid blockade		
Pitressin	0.2,0.3-2.4 (IV) 1.25 (IP) 0.012 (IC)	Rat	Adrenal ascorbic acid depletion[5, 9, 14, 15, 61, 72, 74, 81]
LVP	0.3 (IV)		
Highly purified AVP	0.25† (IP)		
Vasopressin (?)	0.5-1.0 (IP) 0.1		
Pitressin	0.1† (IV)	Rat	Pituitary ATH content (*in vitro* assay)[78]
Pitressin	0.2† (IP)	Rat	P^{32} uptake by adrenals[67]
	Prolonged ether anesthesia		
Pitressin	5.0 (IV,IC)	Rat	Plasma ACTH activity (adrenal ascorbic acid assay)[81]
Lyophilized Pitressin	5.0 (IV)		
AVN-5	5.0 (IV)		
	Morphine-Nembutal block		
Pitressin Highly purified	0.07-0.2†,0.25 (IV) 0.006-.04† 0.08-.40	Rat	Adrenal ascorbic acid depletion, plasma corticosterone[5, 14, 80, 81, 72]
	Chloropromazine block		
Vasopressin (?)	0.1 (IV)	Rat	Adrenal ascorbic acid depletion[67]
	Reserpine block		
Pitressin	0.3 (IV)	Rat	Adrenal ascorbic acid depletion[71]
	Dibenzyline treated		
Vasopressin (?)	0.1	Rat	Adrenal ascorbic acid depletion[72]

TABLE 2. (Continued)

Vasopressin	*Dose U.*	*Animal*	*Index*
	Hypothalamic Lesion		
Pitressin	0.1,0.2,0.61 (IV)	Rat	Adrenal ascorbic acid depletion[14, 54, 55, 78]
Synthetic LVP	0.1 (LS)	Frog	Molting[48]
Highly purified LVP	0.004-.032* (IV) 0.064-.128 (IV)	Rat	Plasma corticosterone[81]
	Pituitary Transplant		
Pitressin Postipofisan	0.01-0.3 (IP,local) 0.3 (IP)	Rat	Adrenal ascorbic acid depletion, eosinopenia[8, 68]
	Decerebrate and Ether Anesthesia		
Pitressin	5.0* (IV)	Rat	Plasma ACTH (adrenal ascorbic acid assay)[82]
	Neurohypophysectomized		
Pitressin	0.3 (SC)	Rat	Adrenal ascorbic acid depletion[71]
	Decapitate		
Pitressin	0.5-5 (IV)	Rat	Plasma ACTH (adrenal ascorbic acid assay)[78]
	Stalk Section		
Pitressin	1 2x3days	Dog	Blood ACTH, Plasma 17-OH corticosteroids, Cytochemical changes in adrenal cortex[45]
	In Vitro		
Pitressin Synthetic LVP Synthetic AVP Highly purified LVP	0.0001 0.00067 0.001 0.0005	Rat	Saffran-Schally assay[79, 84]
Pitressin AVN-2	1.0	Rat	Tissue culture (adrenal ascorbic acid depletion)[85]
Pitressin	1.0-2.0	Rat	Pituitary incubation (adrenal ascorbic acid depletion)[81]

* See Table 1 for footnotes.

Arimura found that the threshold for pituitary activation as measured by plasma ACTH activity fell between 0.04 and 0.08 units of vasopressin in the rat.[8] Nichols *et al.* have noted the threshold to be 0.16 and 0.26 units in the dog. Using highly purified lysine vasopressin in the experiments with unanesthetized dogs, they were able to calculate that a difference of 3.5×10^3 to 7.1×10^3 molecules/second existed between the dose reproducing physiological antidiuresis and that producing ACTH release.[70] Knowing that the threshold for *in vitro* pituitary stimulation is approximately one thousandth of the *in vivo* threshold dose in the rat it can be argued that dilution may account for the necessarily high dose required to produce a sufficient concentration of exogenous vasopressin at the pituitary.[2, 8, 83, 84] However, by the same reasoning, the endogenous vasopressin would have to reach this level to stimulate ACTH release. This would dictate a maximal antidiuresis each time ACTH is discharged. Such a parallel discharge of vasopressin has not been observed experimentally in unanesthetized hydrated dogs[70] sensitive to as little as one or two milliunits of purified lysine vasopressin. In view of this observation and the enormous doses of exogenous vasopressin, it can only be concluded that the physiological significance of the ACTH releasing and adrenal-stimulating potential of the posterior lobe hormone is very hypothetical.

The necessary confirmation of the site of vasopressin activity lies in the demonstration that the posterior lobe hormone has no direct effect upon the adrenal. This was assumed to be clearly established by earlier students of this relationship who found no adrenal activation when the indices of thymic involution, eosinopenia, adrenal weight and content of lipid, cholesterol or ascorbic acid, and peripheral plasma or urinary steroids have been used (Table 3). Using the most direct approach to the problem, Nelson and Hume in 1957 and Hilton in 1959 studied the effect of vasopressin on adrenal venous effluent steroid content. Working with hypophysectomized dogs, Nelson and Hume found that giving 0.2 to 20 units intravenously stimulated corticogenesis.[80] Hilton, using the isolated dog adrenal perfused by hypophysectomized donor blood, found that the addition of as little as 0.00001 μ/ml. to the perfusing blood stimulated corticogenesis.[87] Hilton's apparently logical conclusion that ADH may participate in adrenal activation at the adrenal level may be premature. Lipscomb *et al.* have extended these original observations and have found that such a direct effect of vasopressin on the adrenal is absent in the *in vivo* or *in vitro* rat adrenal.[80] This finding confirms the specificity of ADH for anterior pituitary activation in the rat but does not explain Hilton's observations in the dog. In further studies Lipscomb *et al.* confirmed the responsiveness of the dog adrenal to vasopressin but discovered that the necessary dose was 2×10^4

TABLE 3. THE EFFECT OF VASOPRESSIN IN THE HYPOPHYSECTOMIZED ANIMAL*

Vasopressin	*Dose U.*	*Animal*	*Index*
Pitressin	1.6(IV)†	Rat	Adrenal ascorbic acid depletion (64% block)[74]
Pitressin	5 (IP)†	Rat	Adrenal ascorbic acid depletion (92-98% block)[64, 68]
Pitressin	0.3(SC)† 0.2(IV)† 0.5(SC)†	Rat	Adrenal ascorbic acid depletion (100% block)[14, 71, 82]
Pitressin	0.2(SC)†	Rat	Adrenal ascorbic acid depletion (100% block)[1]
Pitressin Postipofisan	0.3(IP)† 0.3(IP)†	Rat	Eosinopenia (100% block)[62] Adrenal ascorbic acid depletion (99% block)
Pitressin	7.5/15 days(SC)†	Rat	Adrenal weight lipid depletion (100% block)[68]
Pitressin	5 (IP)†	Guinea pig	Urinary neutral reducing lipid (100% block)[60]
Pitressin AVN-5 Synthetic AVP	1-5(IV) 2.5-5(IV) 1-5(IV)	Rat	Adrenal ascorbic acid depletion[78]
Pitressin PNH	1x14 days(IP)† 75x 3 days(IP)† 833x14 days(IP)†	Rat	Adrenal weight, lipid maintenance[80]
Pitressin PNH	1x14 days(IP)† 75x 3 days(IP)† 833x14 days(IP)†	Rat	Adrenal cholesterol depletion[80]
Pitressin PNH	1(IP)† 833(IP)†	Rat	Thymic involution[80]
Pitressin Purified VP Synthetic VP	0.2-20(IV)	Dog	Adrenal vein 17-OH corticoids[89]

TABLE 3. (Continued)

Vasopressin	*Dose U.*	*Animal*	*Index*
Synthetic VP	0.1-0.4 (perf)	Dog	In situ perfusion of adrenal, 17-OH corticoids[87]
Pitressin	0.2† (IP)	Rat	P^{32} uptake of adrenals (64% block)[87]
AVP	0.3† (IV)	Rat	Plasma free corticosterone[89]
Infundin	2x0.02†	Rat	Eosinopenia[18]
Post. Pit. extract	300-600/13 days	Rat	Adrenal hypertrophy[60]
Pitressin	1.0-4.0	Rat	Adrenal ascorbic acid depletion[91]

* See Table 1 for footnotes.

times as large as that reproducing physiologic antidiuresis. They also discovered that the *in vitro* dog adrenal is unresponsive to vasopressin.[50] These studies warrant delay in the acceptance of Hilton's theory of direct adrenal activation by the posterior pituitary.

In another experimental approach Sayers found that the administration of 0.5 to 5 units of pitressin to decapitated animals maintained by artificial respiration resulted in ascorbic acid depletion. This was confirmed in 24-hour hypophysectomized rats in which he injected 1 to 5 units of pitressin and purified or synthetic arginine vasopressin.[81] The significance of these observations is obscure in light of Lipscomb's failure to demonstrate a corticogenic effect of vasopressin upon the rat adrenal.

The coincidence of ADH and ACTH release. The second major tenet of those who have postulated ADH as the mediator of ACTH discharge by the pituitary is that the two hormones are released synchronously and inhibited synchronously. Rothballer appears to have been the first to advance this argument. This investigator based his hypothesis on morphological evidence of depletion of granules (presumably ADH) from the posterior pituitary to stimuli evoking ACTH discharge. Attention was also directed to the similarity of stressors releasing the two hormones.[77]

Mirsky, Stein, and Paulish published a series of experiments in which they observed rapid variations of the antidiuretic activity of peripheral blood following traumatic or emotional stresses. Working with the rat they noted a 120 per cent rise in antidiuretic activity 30 seconds after exposure to intense noise. A similar rise of activity was observed following faradization or intraperitoneal injection of histamine. The administration of cortisone or bilateral adrenalectomy produced changes in the level of antidiuretic substance which paralleled the alterations in ACTH release. In addition they observed that changes in antidiuretic activity occur in hypophysectomized and adrenalectomized animals.[62] These experiments were interpreted as evidence that ADH may mediate ACTH release. The estimates of antidiuretic activity by Mirsky *et al.* have been subject to criticism by Van Dyke who found their assay method to be non-specific.[68] The observations of this group on the relationship between antidiuretic activity of plasma and ACTH release are therefore of uncertain significance.

In an attempt to investigate the possible relationship of the two hormones, Nichols *et al.* simultaneously studied the plasma corticoid and antidiuretic responses of unanesthetized dogs to hypertonic saline injected into the carotid artery. With these more direct criteria for hormone release no correlation was demonstrable between antidiuresis and corticogenesis after endogenous ADH release. Although the antidiuretic response varied from moderate to maximal, elevation of plasma corticoids did not occur. Additional observations made of plasma corticoids during ADH inhibition by hydremia failed to show any parallel between the parameters.[70] MacDonald has noted similar disassociations in the human.[57]

In recent work Dingman and his co-workers were able to selectively stimulate the diencephalon of dogs producing either antidiuresis or ACTH release as indicated by increased corticoid levels in the adrenal effluent blood.[16]

George and Way observed that whereas morphine induced both ACTH and ADH release, only less than one hundredth of the dose was required for stimulation of ADH discharge.[28] They found that the equivalent effect of the larger ACTH-releasing morphine dose could be reproduced with aspirin without any discharge of ADH.[25]

These data appear to justify the conclusion that ADH and ACTH are independently released. The other side of the argument remains to be discussed. Are the two hormones blocked simultaneously? McCann and Brobeck created electrocoagulative lesions of the hypothalamus in rats.[55] These lesions, which inhibited normal adrenal ascorbic acid depletion to stress, interrupted in each case the supraoptic-hypophyseal tract which

Fisher *et al.* had shown to be essential for the release of ADH.[19] The consequent diabetes insipidus in these rats failing to respond to stress by the release of ACTH was taken as strong evidence that ACTH release is mediated by ADH. However, in rats with hypothalamic lesions adrenal atrophy was not observed but rather adrenal hypertrophy which paralleled the severity of diabetes insipidus.[65]

Using plasma corticosterone levels instead of adrenal ascorbic acid depletion as an index of ACTH release, Nichols *et al.* reinvestigated this apparent relationship. In a series of more than 600 rats minute hypothalamic lesions were produced in the area of the median eminence. This lesion inhibited the stress response of plasma corticoids in about one fourth of the animals. These animals also demonstrated diabetes insipidus in all but very few cases. However, when the 303 animals with diabetes insipidus are studied, only half of these demonstrated a block of ACTH release. It is also noteworthy that in this series of animals demonstrating inability to release ACTH adrenal atrophy was observed.[39] The discrepancy between these observations and those of McCann may be due in part to the different indices of ACTH release. However, others using adrenal ascorbic acid depletion as the index of adrenal activation have also noted the lack of correlation between ADH and ACTH blockade.[6,20,23,60] This discrepancy also was noted in humans with diabetes insipidus who demonstrated no impairment of the ACTH response to exercise.[75]

Observing the antidiuretic response in rats, de Wied and Mirsky found that though prednisolone administration effectively inhibited the ACTH release following pain, or the injection of histamine or nicotine, the antidiuretic response to these stresses was intact.[15] In contradistinction, McCann *et al.* noted that hydrocortisone significantly, although incompletely, blocked the antidiuresis following stress.[60] The apparent discrepancy does not eclipse the major agreement that endogenous ADH *can* be released in the animal with effective steriod blockade of ACTH release. Gaunt, Lloyd, and Chart found that hypothalamic antidiuretic activity was depleted by stress and increased by hydrocortisone administration. Parallel changes occurred in the posterior lobe antidiuretic activity except in the hydrocortisone treated animal where the material strikingly accumulated. The changes in activity were reflected in changes in secretory morphology.[24] Schapiro *et al.* studied the urinary 17-OH steroid secretion in guinea pigs and found that steriod production did not parallel the changes in antidiuretic activity of the hypothalamus or posterior pituitary.[66] This evidence speaks against a physiological role of ADH in the regulation of ACTH release and also suggests that the level of endogenous adrenal steroids may

never be sufficiently elevated to produce the ADH inhibiting effect seen with exogenous steroids.

The effects of neurohypophysectomy on ACTH release. A direct approach to the relationship between vasopressin and ACTH release has followed the classic mode of endocrine study: ablation of the posterior lobe. The neurohypophysectomized animal has been found refractory to stresses producing ACTH discharge in normal animals. The results of these studies are summarized in Table 4. One thing apparent from this table is the failure of any investigator to find a complete block of ACTH discharge to all stressors. In order to explain this finding, Nowell has postulated that only "systemic" stresses are inhibited,[71] whereas Smelik and de Wied found only "psychic" stimuli inhibited.[18, 80] Fisher and De Salva noted that removal of the posterior lobe resulted in apparent blockade of the adrenal ascorbic acid response but not the corticosterone response to injected epinephrine.[30]

TABLE 4. EFFECT OF NEUROHYPOPHYSECTOMY ON RESPONSE TO STRESS

Effective stress	*Blocked stress*	*Animal*	*Index of response*
Bell ringing Pitressin Nicotine	Cold Hypertonic saline	Rat	Adrenal ascorbic acid depletion[71]
	Epinephrine Electrical shocking	Rat	Adrenal ascorbic acid depletion[2]
Unilateral adrenalectomy	Cold (diminished) Bell ringing Epinephrine (IM, IV)	Rat	Adrenal ascorbic acid depletion[80]
	"Psychic stimuli" (diminished)	Rat	Plasma free corticosterone[18]
Epinephrine	Epinephrine	Rat	Plasma free corticosterone. Adrenal ascorbic acid depletion[30]
Electrical shocking Splenectomy		Rat	Plasma free corticosterone. Adrenal ascorbic acid depletion[68]

Nichols and Brodish studied both "psychic" and "systemic" stresses in the neurohypophysectomized rat. They observed that the adrenal corticosteroid response to stress was intact. When the adrenal ascorbic acid depletion was

observed in these chronically stressed animals, the results were variable as a consequence of observed elevations in ascorbic acid resting levels. Therefore, it is again emphasized that the ascorbic acid depletion test cannot be used reliably in the intact, chronically stressed rat.[88] Brodish and Long emphasized that the validity of the ascorbic depletion test is limited to the hypophysectomized animal. Its use in intact animals leads to invalid interpretations.[7]

TABLE 5. THE INHIBITORY EFFECT OF VASOPRESSIN UPON STRESS*

Vasopressin	*Dose U.*	*Animal*	*Stress*	*Index*
Vasopressin (DNZ)	0.02-1% (SC)	Mouse	Cold Epinephrine	Adrenal lipid depletion[46]
Pituitrin (DNZ)	0.02-.04% (IV)	Rat	Pain Cold	Adrenal ascorbic acid depletion[44]
Pitressin Synthetic LVP	0.02% (IV) 0.02% (IV)	Rat	Electrical shocking Epinephrine Laparotomy* Unilateral adrenal-ectomy* Immobiliza-tion*	Adrenal ascorbic acid depletion and blood ACTH (ascorbic acid depletion assay)[8]
			Hypertonic saline (SC)	Eosinophil depletion[8]

*See Table 1 for footnotes.

Inhibition of ACTH release by exogenous ADH. Although major emphasis has been given to the stimulatory effects of vasopressin on ACTH release, the material also possesses an ability to inhibit ACTH discharge from the pituitary. The principal results of these experiments are summarized in Table 5. Doses of 0.02 units inhibit various stresses when given to rats and mice 30 minutes before the onset of the stress.[1, 46] The mechanism of this inhibition has been extensively studied by Arimura. This investigator found that the timing of the pre-medication was critical, with deviations from the 30-minutes-before-stress schedule failing to inhibit the stress response. Synthetic lysine vasopressin and pitressin were found to be equally effective. With the dosage employed, there was no change in the hematocrit or in the pituitary portal vessels when under direct observation. The phenomena could not be the result of a refractory period following the administration

of the premedication for small doses of epinephrine administered 30 minutes before stress did not inhibit the response. Inactivated lysine vasopressin was also without effect.[8]

The physiological role of these phenomena is not apparent but greater significance should be attached to these observations for they occur with doses of vasopressin which as Kurokawa has noted approach the physiological level.[48]

Itoh *et al.* found that hydrated rats were more responsive to stress and that dehydration reduced the adrenal response to the same stresses: epinephrine, histamine, and cold. Under these conditions, no change in adrenal sensitivity could be detected.[41] Arimura observed that dehydration of rats suppressed their response to electrical shocking and unilateral adrenalectomy. In his hands hydration seemed to also suppress ACTH release although producing hypertrophic adrenals.[3] These observations in general support the thesis of these investigators that ACTH may be inhibited by ADH.

The significance of the ADH theory of ACTH release. In response to the tenets of those who postulated that the antidiuretic hormone might be the mediator of ACTH release, one can now reply that there is no parallelism between release or inhibition of the two hormones. Only pharmacological doses are capable of stimulating ACTH release, and the integrity of the posterior pituitary or its hypothalamic projections is not necessary for ACTH release.

What then is the significance of all the studies on ADH and ACTH interrelationships? The evidence at hand does not exclude vasopressin from the cast of supporting players. Just as the earlier theories of epinephrine release and peripheral utilization of corticoids have been demonstrated to be insufficient in their attempts to explain the full phenomenon of ACTH release; the ADH theory cannot account for the observed phenomena when rigorous criteria are used. Just as epinephrine and push-pull theories have remained part of a background of well-documented factors regulating the pituitary-adrenal axis, the "vasopressin theory" will form part of our understanding of ACTH physiology.

Several conclusions remain unquestioned about the relationship of the antidiuretic hormone to ACTH release. The first is the ACTH releasing activity of a large variety of preparations of the pressor material in a variety of animal preparations as observed by all of the available criteria for ACTH release (Table 1). The further observations that the ratio of ACTH releasing activity to pressor activity is constant regardless of purification,[40, 74] the studies with purified or synthetic vasopressin, and the parallel

inactivation of these activities,[9] indicate that the activity is not due to a contaminant. The action of vasopressin in animals blocked to stress indicates a specificity of the effect on ACTH release (Table 2). These phenomena are valuable contributions to an expanding field of pharmacology of peptides.

Another contribution of these studies is that they may give insight into the nature of the physiological corticotrophin-releasing factor. It is tempting to speculate, as has Guillemin, that the activity of exogenous vasopressin may be due to a structural relationship to the physiological mediator of ACTH release.[87, 88, 89] Indeed Schally *et al.* have found that their ACTH releasing factor is closely related in size and amino acid composition but lacking in vasopressin activity.[85]

Although the evidence seems conclusive that ADH is not essential for the stress response of ACTH, there are several indications that it may play a more subtle role in regulating this anterior lobe hormone. That there is a relationship between the pituitary-adrenal system and ADH is supported by the excellent work of Gaunt *et al.* who found that hypothalamic antidiuretic activity varied inversely with plasma cortical hormone content.[24] The studies of pituitary ACTH content by Fortier[21, 22] reveal that changes in ACTH concentration parallel the hypothalamic antidiuretic activity observed by Gaunt.

The inhibitory effect of ADH on ACTH release as demonstrated by Arimura and other Japanese investigators may be another factor in the background of the ACTH release story (Table 5). The fact that this phenomenon is demonstrated with near-physiological doses and seems to be quite specific in activity supports such a concept.

With these qualifications in mind it seems justified to relegate the ADH theory of ACTH release to the emeritus role enjoyed by previous attempts to explain this neural-glandular link in ACTH release. The contributions of this theory have been both material and intellectual for it has stimulated much of the present work in this field. Is not this sufficient justification for these studies?

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EFFECT OF TRIIODOTHYROPROPIONIC ACID ON PLASMA LIPIDS AND BUTYL EXTRACTABLE IODINE†

INTRODUCTION

The discovery that various of the naturally occurring and synthetic analogues of thyroxine possess some of the many physiological properties of thyroid hormone in variable activity[9,10,12,14,16,17,25,28] has stimulated a search for an analogue with the cholesterol-lowering action of desiccated thyroid but without toxic side effects. Such a compound has been sought to decrease the serum cholesterol concentrations of patients with hypercholesterolemia and vascular disease. Thyroxine itself lowers the serum cholesterol but the effect is sustained only when the dosage is raised to concentrations which may produce tachycardia, nervousness, palpitations, or weight loss.[29] Bauer, McGavack, and Swell[5] and Rawson, *et al.*,[21] reported a reduction of serum cholesterol in man without increase in basal metabolic rate by the administration of 3, 5, 3′-triiodothyropropionic acid, an analogue of thyroxine referred to here as TRIPROP. Other lipid parameters were not measured, and long-range studies have not been reported.

The present study was undertaken to determine the effect of TRIPROP‡ on serum lipids when administered to normal persons in large doses for a short period of time and when given for longer periods to patients with elevated concentrations of serum lipids. The effect of TRIPROP on serum

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triglyceride concentration was of particular interest in relation to the increased serum triglycerides in coronary artery disease.[3,4] In short-term experiments, large amounts of TRIPROP were given to obtain a maximal effect of the drug on serum lipids, particularly on plasma-free fatty acids, a fraction of plasma lipids which has been found to be important in mobilization of fat from depots.[7-9] In the long term experiments small initial doses of TRIPROP were increased gradually to avoid possible toxic effects to patients with overt or incipient coronary artery disease.

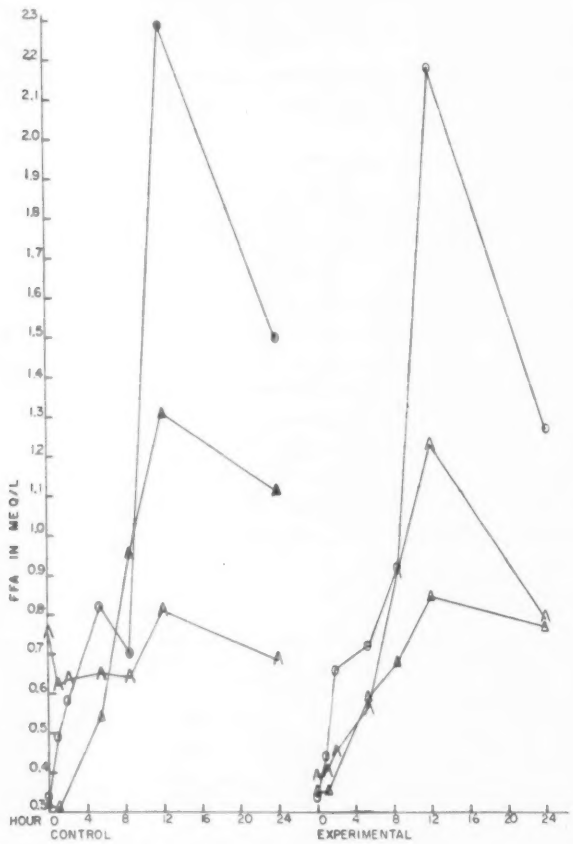

FIG. 1. Plasma FFA of three subjects during a control day (left) and following the administration of a single dose of 0.3 mg. per kg. bodywegiht of TRIPROP.

METHODS

Acute experiments. (i) The effect of short-term administration of TRIPROP was studied in medical students or hospital employees with an age span of 23 to 26 years. All were free from known disease. The effect of a single large dose was assessed in three normal persons. Measurements were made of the plasma FFA,[7, 20] total fatty acids,[2, 18] lipid phosphorus,[27] and total cholesterol[1, 2] at about 7:30 A.M. after an overnight fast of approximately twelve hours. Samples were taken at 1, 2, 5½, 8½, 12, and

TABLE 1. BEI CONCENTRATIONS IN PLASMA OF FOUR ADULTS AND ONE 9-MONTH-OLD BABY AT INTERVALS FOLLOWING A SINGLE DOSE OF TRIPROP

		BEI in μg. per 100 ml. serum												
Subject	*TRIPROP Dosage*	*Before TRIPROP*	*Hours after TRIPROP* ¼	1	2	4	5½	6	8½	12	24	56	94	192
A.M.	0.15 mg./kg. (17 mg.) with 50 Gm. glucose	4.6*		6.6*			10.9*			9.8*		5.3	3.0	
"	0.15 mg./kg. with regular breakfast (17 mg.)	3.7		14.8			8.7			9.5	7.5	2.5	3.5	4.1
V.M.	0.3 mg./kg. (24 mg.)	3.8*		12.0*			19.1*			11.3*	11.3*			
P.L.	0.3 mg./kg.	4.2*					16.7*							
C.P.	3 mg.	4.7*	6.2*	5.9*							4.6			
Pod.	4 mg.	3.0**			17.0	12.6		11.5						

*Fasting (except for the glucose in the first experiment on A.M.).
**Average of three previous determinations.

24 hours for the analysis of the FFA, at 8½ and 24 hours for the lipid phosphorus, total fatty acids, and cholesterol. Triglycerides were estimated by difference[2, 18] and were expressed as triglyceride fatty acids in milliequivalents per liter. At 5½, 8½, and 24 hours, exhaled gas samples were obtained for determination of the respiratory quotient and the basal metabolic rate.[6, 24] Because of the method of gas collection, any degree of hypoventilation or hyperventilation would give rise to a misrepresentation of the actual amount of carbon dioxide produced and oxygen used by the body's overall metabolism. As a result of these uncontrollable factors, the results of the BMR and respiratory quotient measurements in the present study cannot be accepted as unequivocal. Throughout the 24 hours the subjects did not ingest any food but were allowed to have water.

One week later the same measurements were made as on the control day, but after the initial collection of samples, TRIPROP in doses of 0.3 mg. per kilo body weight was administered orally. In addition to the above measurements, at 0, 1, 5½, 8½, 12, and 24 hours in one subject and at 0 and 5½ hours in another, blood samples were obtained for the analysis of the serum butanol extractable iodine (BEI).[13]

(ii) An independent study on the influence of a single dose of TRIPROP on the concentration of the BEI was made on three normal subjects, on a patient with coronary artery disease, and on a nine-month-old baby.

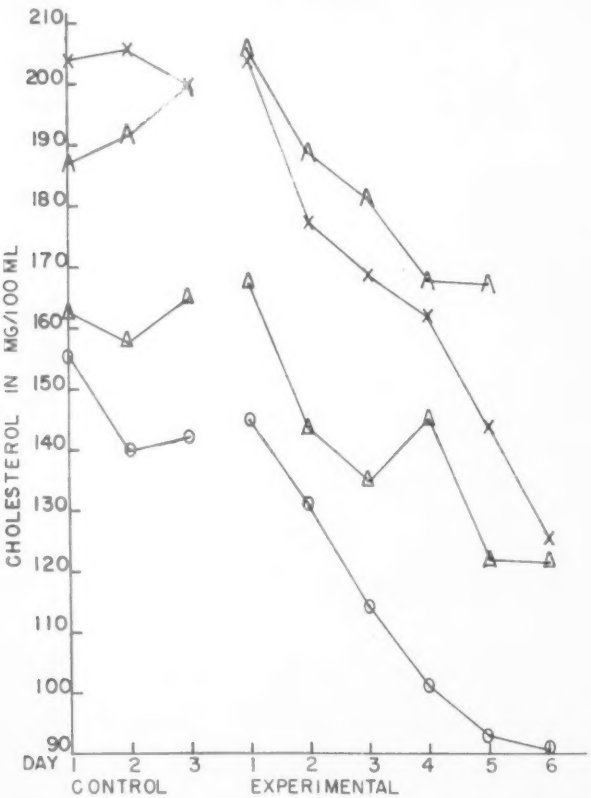

FIGURE 2. Plasma cholesterol of four subjects during a control period and during a five-day period of daily administration of 0.3 mg. TRIPROP per kg. body weight. Medication was begun immediately after blood was drawn on experimental day one, and given each subsequent day after the withdrawal of blood for lipid analysis.

Chronic experiments. Six euthyroid patients with elevated concentrations of serum cholesterol, triglycerides, or both, and one obese myxedematous patient were given TRIPROP in doses varying from 1 to 18 mg. daily. The obese patient was on a calorically restricted diet. The fat intake of the other patients was moderately restricted and did not change throughout the period of study.

In vitro experiments. In order to interpret the BEI's, *in vitro* recoveries from serum of several analogues of thyroxine including TRIPROP were conducted. The theoretical BEI was calculated from the known composition of the various compounds and the previously determined BEI of the sera.

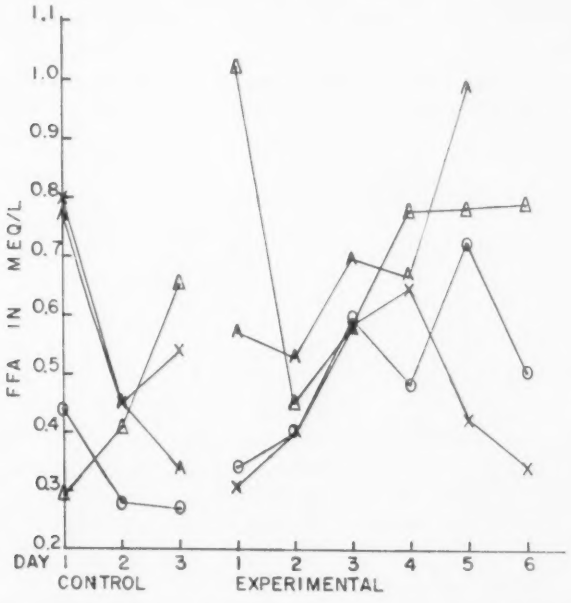

FIG. 3. Plasma FFA of four subjects during a control period and during a five-day period of daily administration of 0.3 mg. TRIPROP per kg. body weight. See legend of Figure 2 for time of medication.

RESULTS

Acute experiments. Since no definite or consistent changes in concentration of cholesterol, lipid phosphorus, triglycerides, or respiratory quotient were observed during the 24 hours following the ingestion of a single large dose of TRIPROP, such data are not presented. The FFA increased to the same degree with fasting whether or not TRIPROP was given (Fig. 1).

This dose of TRIPROP caused a marked increase in BEI within one to five hours, following which the BEI gradually decreased to a point below the pretreatment concentration within 56-94 hours (Table I). In subject A.M., a more rapid rise occurred when TRIPROP was administered with a regular meal than with a solution of glucose.

Daily administration of TRIPROP for six days caused a considerable reduction of plasma cholesterol to a concentration varying between 62 and 82 per cent of the initial values (see Fig. 2). However, the plasma total fatty acids, triglyceride fatty acids, lipid phosphorus, and cholesterol-phospholipid ratios did not exhibit a sufficiently consistent response to the administration of TRIPROP to permit presentations of data. The rise of FFA was difficult to interpret because of the variability of this fraction during the control period (Fig. 3). The BEI rose markedly during the six days (Table 2). The basal metabolic rate was variable and open to errors mentioned in methods, but nevertheless it appeared to rise during the administration of TRIPROP (Table 3).

TABLE 2. EFFECT OF 0.3MG. TRIPROP PER KG. BODY WEIGHT PER DAY ON PLASMA CONCENTRATIONS OF BEI IN μG. PER 100 ML.*

	SUBJECT	
Days of TRIPROP Ingestion	*E.L.*	*M.W.*
0	3.2	4.8
2		28.3
3	22.2	
4		19.9
5	24.4	

*A total of 19 mg. TRIPROP daily was given to E.L., and 14 mg. to M.W.

TRIPROP did not exert any definite or consistent influence on either systolic or diastolic blood pressure. The morning pulse rate, however, showed an upward trend. After five daily doses of TRIPROP the various pulse rates were from 14 to 26 beats per minute more than the initial values. All four subjects lost weight on the six-day regimen. In two subjects, as much of a reduction as seven pounds in five days was seen. Mild and transient headaches occurred during the six-day experiment in two subjects, in one of whom the onset of cephalalagia coincided with the onset of menstrual flow, and with temporary increase in perspiration in the other of the two subjects. With these exceptions, TRIPROP in the large dosage given here

did not seem to cause any symptoms of nervousness, jitteriness, irritability, increase in appetite, palpitation, or diarrhea.

Chronic experiments. Table 4 summarizes the serum lipids and BEI's of six euthyroid patients and one myxedematous patient given TRIPROP in various doses. Of the euthyroid patients, four or five patients receiving one or two miligrams of TRIPROP daily had no significant change in cholesterol. The first patient in Table 4 voluntarily lost weight during this period. A decrease in cholesterol happened to coincide with the weight loss as sometimes occurs temporarily. Serum cholesterol concentrations decreased in

TABLE 3. EFFECT ON BASAL METABOLIC RATE OF FOUR SUBJECTS GIVEN TRIPROP IN DAILY DOSES OF 0.3 MG. PER KG. BODY WEIGHT, FOR SIX DAYS. BASAL METABOLIC RATE—COMPARISON IN PERCENTAGE WITH STANDARD VALUES FOR COMPARABLE AGE AND SEX.

	Control			*Experimental*					
	Day 1	Day 2	Day 3	Day 1*	Day 2	Day 3	Day 4	Day 5	Day 6
R.M.	+2.36	—15.47	—3.45	—0.54	+46.6	—19.79	—4.48	+11.27	——
W.R.	+14.1	+4.9	+16.4	+3.30	+32.8	+13.03	+35.80	+18.01	+30.7
E.L.	—34.88	—13.56	—3.04	—25.59	—8.62	+2.30	+1.00	+9.0	+8.4
M.W.	+9.26	—3.26	——	—2.36	+3.29	+13.4	+15.0	+21.6	——
Average	—5.3	—9.4	+4.0	—6.5	+18.5	+2.4	+11.7	+15.0	+19.0

*Subjects began taking TRIPROP after collection of all specimens on day 1 of experimental period.

three of five patients receiving three to four mg. daily (43-57-41, A.L., K.T.) and in four of five patients given six to eight mg. daily. One patient, C.M., who had responded minimally to lower doses, was given 9 to 12 mg. TRIPROP. On this dose her cholesterol fluctuated about a slight but insignificantly lower concentration (93 per cent of the pretreatment concentration). A dose of 9 mg. was selected as maintenance since she reported that she felt jittery when receiving 12 mg.

The myxedematous patient, 49-46-20, was treated with a starting dose of 4 mg. TRIPROP daily. The cholesterol dropped progressively from 453 to a low of 204 mg. per 100 ml. when the dose was 12 milligrams, eight weeks after starting therapy. Serum cholesterol increased somewhat despite further increase in the dose of 18 mg. daily. Because of excessive perspiration, the amount of drug administered was reduced to 12 mg. daily. After 18 months of continuous medication at this dose, the cholesterol was 242 mg. per cent.

The response of triglycerides was quite variable, with no consistent trend,

TABLE 4. EFFECT OF VARIOUS DOSES OF TRIPROP ON CONCENTRATIONS OF SERUM CHOLESTEROL TRIGLYCERIDES, BEI, CHOLESTEROL: PHOSPHOLIPID RATIO, AND ON BODY WEIGHT OF SEVEN PATIENTS

	TRIPROP		Cholesterol			Triglyceride fatty acids				BEI				
Patient hosp. no., age & sex	Dose mg. daily	Duration of dosage	No. of determinations	Mean mg./100 ml.	Range	No. of determinations	Mean mEq/l	Range	C/P ratio	No. of determinations	Mean μg./100 ml.	Range	Weight lbs.	Comment
H.D.	0	—	1	224		1	7.6		1.02	1	5.3		183	Coronary two
—	1	4 mo.	2	185	(168-202)	2	6.5	(4.8-8.2)	0.82	1	4.3		175	years ago
50 M	0	3 wks.	1	246			4.8		0.99	1	3.9		176	
	0	3 mo.	1	248			7.4		0.92	1	—		181	
	0	—	2	320	(309-330)	2	6.3	(5.0-7.6)	1.0 (.94-1.05)	1	3.9		177	Coronary
43-57-41	1	7 mo.	7	316	(300-349)	7	7.4	(2.9-12.8)	1.0 (.90-1.12)	5	3.9	(3.2-5.0)	177	three years
54 M	2	1 mo.	1	318		1	6.7		1.10	4	2.9			ago; xanthel-
	4	4 mo.	4	278	(229-299)	4	7.2	(6.1-9.7)	1.01 (.93-1.14)	2	3.3	(2.0-4.7)	177→172	asma, tendon
	6	7 mo.	5	282	(264-320)	5	5.9	(3.6-7.5)	1.02 (.89-1.16)	1	6.2	(6.0-6.4)	171	xanthomata.
	0	1 mo.	1	355		1	7.2		1.03	1	4.9		177	
	0	2 mo.	1	338		1	4.4		1.07		—		178	
	6	4 mo.	1	303			4.3		—		—		172	
	6	7 mo.	1	296		1	5.9		—		—		172	
C.M.	0	—	3	652	(620-682)	1	1.3		1.60	2	4.5	(3.0-5.9)	115-120 past 5 yrs.	Tendinous
—	0	Thyroid 2 gr.	5	661	(512-734)	2	2.5	(1.0-3.9)	1.38	3	4.7	(3.2-5.6)	115-120 past 5 yrs.	and cutaneous xanthomatosis
48 F		10 yrs.												
	1	Thyroid 2 gr. 1 mo.	1	600		1	9.0		1.32	1	5.5		116	
	6	(no thyr.) 1 mo.	1	698			6.7		1.36	1	4.6		118	
	9-12	22 mo.	13	604	(526-674)		5.0	(8.5-1.5)	1.42	10	4.9	(3.8-6.7)	113→100	

		0	(2&0mo.	2	299	(260-350)	2	6.4	(6.3-6.5)	0.99		1	5.8		115	
B66833			before Rx)													Angina
61	F	1	7 mo.	5	292	(260-327)	5	6.9	(4.1-10.0)	1.08		4	3.7	(2.9-4.2)	112-109	
		3	2 mo.	1	311		1	3.9		1.08		1	4.6			
		6	4 mo.*	4	253	(217-271)	4	5.0	(3.7-7.0)	0.87	(0.80-0.96)	1	6.0		101	
		0	1 wk.	1	296		1	4.5		—		1	3.4			
			2 wks.	1	260		1	10.1		—			—			
			3 wks.	1	288		1	7.7		—			—			
A.L.		0		4	398	(327-443)	4	44.4	(32.5-66)	—		1	2.4		222→215	Essential
—		on T_3 or thyroid		2	286	(284-287)	2	19.0	(12-25.7)	0.93			—			hyperlipemia
50	M	2	1 mo.	1	381		1	33.9		0.85		1	2.5			
		4	1 mo.	1	266		1	27.6		0.83		1	3.7		216	
		6	2 mo.	2	261	(256-265)	2	21.0	(19.4-22.8)	0.93		1	5.1		206	
		0	6 mo.-1 yr.	2	346	(307-386)	2	40.0	(34-46)	0.97			—		215	
K.T.		0		2	307	(319-294)	2	5.1	(4.9-5.3)	1.08	(1.08-1.08)		—		181	Coronary
—		3	3 weeks	3**	269	(265-275)	3	4.4	(2.7-5.9)	1.00	(0.99-1.02)		—		178	one and five
43	M	6	2 weeks	1	244		1	4.5		1.01			—			years ago
		0		2	463	(451-454)	1	17.6		—		2	1.0	(0.5-1.5)	330	Obese,
49-46-20		4	1 week	1	364		1	17.1		—		1	1.6			hypothyroid
56	F	6	1 week	1	272		1	13.2		—			—			
		8	1 week	1	246		1	6.4		—		1	2.5		325	
		9	1 week	1	232		1	9.8		—		1	5.7		323	
		12	4 weeks	1	204		—	—		—		1	6.3		316	
		18	6 weeks	1	230		1	9.5		—		1	3.7		312	
		12	1½ yrs.	1	241		1	13.2		—		1	4.2		270	

*4 months without drug preceded this dose. **average of weekly determinations.

as indicated in Table 4. Only one of the seven patients, C.M., showed a decrease in the cholesterol-phospholipid ratio while receiving TRIPROP.

In general, BEI's of the euthyroid patients were in the normal range of 3.3 to 6.4 micrograms per 160 ml. both before and during treatment. Exceptions were the slightly low BEI's before treatment in two patients (A.L. and C.M.); however, at other times these patients had normal BEI's while not receiving medication. There was a tendency for the BEI's to be lower

TABLE 5. SERUM BEI AND LIPIDS AT INTERVALS DURING THE DAY IN A PATIENT TAKING A DAILY DOSE OF 5 MG. TRIPROP AT BREAKFAST AND 4 MG. AT LUNCH. LAST COLUMN SHOWS STUDY MADE 48 HOURS AFTER LAST MEDICATION

	9 a.m. No TRIPROP after previous noon	*11 a.m. 2 hrs. after 5 mg. TRIPROP*	*2 p.m. 2 hrs. after 4 mg. TRIPROP*	*48 hrs. after last TRIPROP*
BEI, μg./100 ml.	3.8	10.5	13.6	1.4
Cholesterol, mg./100 ml.	614	609	633	822
Triglyceride fatty acids, mEq./L.	4.0	8.5	5.2	7.4
Cholesterol: phospholipid ratio	1.40	1.26	1.35	1.60

than the pretreatment values when the patients were receiving small doses (Table 4) and to be in the upper range of normal, up to 6 μg. per 100 ml., on higher doses. It should be noted that BEI's were determined at least 12 hours after the last medication. Considerably higher values occurred shortly after intake of TRIPROP, as shown in Table 5. This table shows a normal BEI in the morning when no TRIPROP had been administered for 21 hours, but it shows a high value later in the day, a few hours after the patient had taken her TRIPROP. Forty-eight hours after temporary withdrawal of the drug, the BEI was in the hypothyroid range and the cholesterol had risen to the highest value ever recorded for this patient. She felt rather tired and let down at this time.

All patients lost weight on effective and sometimes on ineffective doses (Table 4). Patients rarely had resting pulse rates greater than 80 beats per

minute, and none displayed a rise in blood pressure while receiving the drug. Increased perspiration, jitteriness, and easy fatigability were infrequent complaints and noted only during the administration of doses of 6 mg. or greater. Occasionally, faint pruritic rashes were observed but disappeared during the continued treatment. For the most part, there were no subjective symptoms.

TABLE 6. RECOVERY OF VARIOUS ANALOGUES OF THYROXINE AS BEI FROM SERA TO WHICH ANALOGUES WERE ADDED IN KNOWN AMOUNTS*

Analogue	*Serum BEI*	*Theoretical BEI (Serum BEI plus iodine added in this analogue)*	*Iodine recovered as BEI*
	micrograms per 100 ml.		*micrograms per 100 ml.*
Tetraiodothyropropionic Acid	4.5	7.3	8.0
Triiodothyroacetic Acid	3.3	9.4	9.3
Tetraiodothyroacetic Acid	3.7	9.9	9.9
Triiodothyropropionic Acid	3.2	9.8	9.4
Triiodothyronine	3.9	7.9	8.0

* Analogues were supplied by the Warner-Chilcott Laboratories Division. The solution of analogue in serum was of such concentration that half of 1 ml. of analogue solution was added to portions of serum varying in quantity from 4 to 8 ml.

In addition, solutions of each of the analogues above except triiodothyronine were subjected to total iodine analysis. Recoveries agreed within 100 to 107 per cent of the calculated total iodine content.

In addition to the seven patients listed in Table 4, three obese patients given TRIPROP with the purpose of inducing weight loss did not display any weight-reducing effect from as much as 18 mg. daily. It was necessary for them to restrict calories in the same manner as obese patients not receiving the drug.

In an isolated study not included in the tables, TRIPROP was given for three days in doses of 4 mg. daily to a 28-year-old euthyroid female patient with an enlarged thyroid. Such intake of TRIPROP decreased the uptake of a tracer dose of radioactive iodine from the upper normal range

to a borderline low range, and produced an increase in the BEI from 6.1 to 9.0 μg. per 100 ml. With continuation of the drug, size of the thyroid gland decreased definitely.

In vitro studies. Recoveries of tetraiodothyropropionic acid, triiodothyroacetic acid, tetraiodothyroacetic acid, triiodothyropropionic acid, and triiodothyronine added *in vitro* to serum were virtually complete (Table 6).

DISCUSSION

The striking reduction of plasma cholesterol following the administration of TRIPROP in the six-day experiment indicates that TRIPROP possesses the cholesterol-lowering action of desiccated thyroid.[20] Bauer, McGavack, and Swell, in a recent paper, reported that the administration of TRIPROP in a dose of 6 mg. daily to euthyroid adults caused an average reduction in serum cholesterol of 25 per cent after a period of two to four weeks.[5] However, in the present investigations (Fig. 2), a dosage of TRIPROP much larger than that used by Bauer, *et al.* was adopted and correspondingly, the response was observed much sooner. The brevity of the acute experiment prevents prediction of the response of serum cholesterol to a more prolonged treatment with TRIPROP at the constant dose of 0.3 mg. per kilo of body weight.

The long-term administration of TRIPROP to patients with initially elevated serum lipids resulted in a small reduction in concentration of cholesterol when administered in doses varying from 3 to 9 mg. daily. The effectiveness of the drug appeared to depend on a time-dose relationship. If a dose sufficiently great to lower the cholesterol was achieved within four months of initiation of TRIPROP, the cholesterol decreased more than 15 per cent. If an effective dose was reached after six months or longer of gradually increasing small doses, the cholesterol was reduced by less than 10 per cent. The only instance of a marked and sustained reduction in concentration of cholesterol was for the myxedematous patient in whom TRIPROP, in doses from 9 to 18 mg. daily, appeared as adequate as desiccated thyroid in the treatment of hypothyroidism. Possibly smaller doses would have served as well[21] but large doses were given to encourage weight loss.

Although this series is small, a comparison of the acute experiments, not only with short term experiments of others but also with the long term evaluation of TRIPROP, is compatible with an escape of the cholesterol-lowering effect of TRIPROP administered to euthyroid persons. Such escape might be expected from the infrequency with which low serum choles-

terol concentrations are found in naturally hyperthroid persons although the reverse finding of high cholesterol in hypothyroidism is the rule.[19]

Escape of the cholesterol-lowering effect of TRIPROP may be accounted for in part by suppression of endogenous thyroid function occurring early after administration of the drug. This suppression was manifest in a single instance by a marked decrease in I^{131} uptake in a patient after three days of receiving TRIPROP; in normal subjects, by a decline in concentration of BEI below pretreatment values three to four days after a single large dose (Table 2); and, despite the continued administration of TRIPROP in large doses (Table 2), by a decrease of the fourth day BEI after an initial rise. Additional evidence for the suppression of endogenous thyroid function was the tendency for BEI's to be low in patients receiving small doses of TRIPROP (Table 4). A similar phenomenon occurred to a more marked degree in one patient who temporarily stopped the drug and who 48 hours later had a BEI in the hypothyroid range and a marked elevation of her cholesterol (Table 5).

The PBI but not the BEI was greatly elevated during administration of TRIPROP. One patient, 49-46-20 (Table 4) had a serum PBI[20] of 22 μg. per 100 ml. on two occasions when the BEI was in the normal range and the patient was clinically euthyroid. PBI's in a similar range and also out of proportion to the clinical response were reported by Hill, *et al.*[13] in patients receiving TRIPROP and tetraiodothyropropionic acid. BEI's during such treatment have not previously been reported. The complete recovery of TRIPROP added to serum *in vitro* (Table 6) excludes the possibility that incomplete recovery of TRIPROP accounted for the discrepancy between BEI and PBI.

Weight loss was noted in all subjects of the acute and long-term experiments. No consistent changes in diet were reported by any of the patients, although in studies of outpatients exact caloric intake is difficult to judge. The well-known increase in basal metabolic rate characteristic of the hyperthyroid state could, if induced by administration of TRIPROP, cause weight loss. The present study shows a suggestive increase in the BMR following the acute administration of TRIPROP but variability of the parameter prevents firm conclusion. At the doses given, it is possible that TRIPROP caused weight loss by increasing the basal metabolic rate. Others have reported a calorigenic effect of TRIPROP and have also found that the cholesterol-lowering effect of TRIPROP occurs at lower doses than the calorigenic effect in myxedematous and euthyroid patients.[5,12,21] At the doses used in the present study the cholesterol-lowering and weight-reducing action of TRIPROP appeared to coincide. Indeed, with prolonged adminis-

tration, its effect on weight was manifest at a time when the cholesterol effect was minimal.

Rich, Bierman, and Schwartz[20] have recently demonstrated that the hyperthyroid state, whether as a result of hyperthyroidism or as a consequence of administration of triiodothyronine, is accompanied by a rise in the concentration of plasma FFA. The rise in plasma FFA was thought to signify mobilization of fat as FFA from adipose tissue. The present experiments, however, show only suggestive increases in the values of FFA following administration of TRIPROP in doses as large as 0.3 mg./kg. of body weight. The lack of a clear-cut effect of thyroid-like hormones on FFA could be explained by two of its opposing actions as proposed by Hagen.[21] Thyroid hormone increases the sensitivity of adipose tissue to insulin, causing an increased utilization of glucose with resulting suppression of FFA release. At the same time thyroid hormone exerts a direct fat-mobilizing effect on adipose tissue with an increase of FFA release. A balance of these two actions could result in no over-all change in FFA concentration.

The evidence presented here is compatible with the thesis that TRIPROP has some of the physiological properties of thyroid to a greater extent than others. Both the cholesterol-lowering and weight-reducing effects of TRIPROP appear to be similar to those of desiccated thyroid. However, the cardiovascular effects of thyroxine were notably absent.

Two of the four patients with previous myocardial infarctions had episodes of myocardial insufficiency while receiving the drug, although the attacks could well have been the result of the disease and not of the drug. Two patients with tendinous and skin xanthomata exhibited progression of their lesions during two years of therapy with the drug, perhaps because effective dosage with regard to its effect on the cholesterol concentration was not maintained during all of this period.

With respect to cholesterol concentrations, the dramatic reduction in the acute experiments was not sustained with prolonged use of the drug. Serum triglycerides, which may be of greater significance than cholesterol in coronary artery disease,[5, 6] were not consistently influenced by TRIPROP administration. This accords with the smaller response of low density lipoproteins, the main source of plasma triglycerides, rather than the response of cholesterol to the administration of thyroid hormone, and it is in accord with the usually normal concentration of triglycerides in the sera of hypercholesterolemic hypothyroid patients.[19] The possible therapeutic usefullness of TRIPROP in creating and maintaining an altered *in vivo* lipid environment cannot be determined on the basis of the present study.

SUMMARY

1. The effect of 3,5,3' triiodothyropropionic acid (TRIPROP) on plasma lipids was studied by the administration of this substance to seven normal subjects in a short-term experiment, to six euthyroid patients with elevated lipid concentrations in a long-term study, and to one myxedematous patient. Serum or plasma was analyzed for BEI, cholesterol, FFA, total fatty acids, triglyceride fatty acids, and phospholipids.

2. No consistent effects of TRIPROP on plasma FFA, triglyceride fatty acids, phospholipids, cholesterol, and basal metabolic rate were observed within 24 hours of a single large dose although a marked increase in plasma BEI took place within one to five hours. However, five days of administration of 0.3 mg. per kg. body weight caused a marked decrease in serum cholesterol concentration varying from 18 to 38 per cent. The changes in FFA were equivocal, and the other lipid components did not change. Weight loss occurred in all subjects. The subjective symptoms of thyroid overactivity such as tachycardia, palpitations, and nervousness were absent.

3. The administration of 3 mg. or more of TRIPROP daily to six euthyroid patients with high blood lipids caused a small reduction of serum cholesterol. The decrease was greater than 15 per cent during the first four to six months of treatment only, and was less than 10 per cent with prolonged administration. No consistent effect on triglyceride concentration or the cholesterol-phospholipid ratio was noted. Weight loss occurred in all patients given doses sufficient to lower the cholesterol. Subjective symptoms were absent or minimal.

4. Marked and permanent reduction of all lipid components of serum and restoration of the euthyroid state followed administration of TRIPROP to one myxedematous patient.

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MICROANGIOGRAPHIC STUDIES OF KIDNEY AND BRAIN IN ANIMALS: TECHNICAL ASPECTS†

The application of X-rays to microscopic study of thin specimens was first attempted by Heycock and Neville[10] in 1897, two years after the discovery of X-rays. This was followed in 1913 by Goby's[7] demonstration of microscopic details of small biological samples by radiographing the thin specimen in close contact with a photographic plate. The negative was subsequently enlarged photographically. He introduced the term, microradiography, and in 1925 succeed in producing stereomicroradiographs.[8] In recent years, microradiography has progressed along two lines: the quantitative analysis of cell mass and of cellular molecular constituents, and the qualitative investigation of morphology, pathology, and physiology of biological systems, especially with the aid of radiopaque contrast media.

As early as 1935, Grechishkin and Prives[9] in Leningrad attempted to visualize small blood vessels in bone with microradiographic technique, using Thorotrast‡ as a contrast medium. They were unable to define vessels of capillary size because of the coarse-grained emulsion they used. Bohatyrschuk[4] in Kiev in 1944, using a fine-grained emulsion and a Bucky X-ray tube, demonstrated capillaries with Thorotrast in sections of mammalian organs and tumors. The British school under Barclay[1,2] in 1947-1949 used a sealed-off diffraction X-ray tube and a variety of contrast media including Thorotrast, colloidal silver iodide, colloidal metallic bismuth, and Neoprine Laxtex¶ in the study of minute vessels in surgical and autopsy specimens. In this country, Tirman and associates[12] produced technically good micro-

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‡ Now available as 25 per cent suspension of thorium dioxide, supplied by Hayden Chemical Co., New York.

¶ Neoprine Laxtax—supplied by B. B. Chemical Co., Ltd., Leicester, England.

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radiographs of small blood vessels of the rabbit kidney and other organs using colloidal silver iodide with gum acacia.

The term "microangiography" was first introduced by Bellman[8] in Sweden in 1953 and corresponds to "angiography" as used in clinical diagnostic radiology. He also applied microangiographic technique to the study of living tissues, but his work was limited to thin organs such as the rabbit ear, or intact organs during a surgical operation. Saunders[11] in Canada in 1957 elaborated upon this technique and prepared various muscular tunnels, flaps, and windows to permit the introduction of a photographic plate beneath muscle in living animal. He used Micropaque* as a contrast medium and was able to demonstrate vasoconstriction and vasodilation in skin following drug administration.

The purpose of this paper is to present a simple technique of microangiography with which detailed vascular structure can be studied under normal and various pathologic conditions. The brain and kidney were selected because of their characteristic vascular patterns, which are thought to be highly vulnerable to radiation and other injuries.

MATERIALS AND METHODS

The X-ray source. A fundamental aspect of microradiography is selection of proper wave lengths of X-rays for examination of specimens of various types of materials. The rule of thumb is that the higher the resolution required, the thinner must be the specimen and the "softer" the X-ray beam. In examination of blood vessels, a relatively thick section is desirable so that a volume pattern of the blood vessels may be seen and the bulk of contrast medium will not leak from the vessels during sectioning. The optimal tissue section for microangiography is about 100 to 300 μ in thickness. According to Engström's[6] calculation, the most suitable X-ray wave length lies between 1 and 10 Å, to be generated at about 10 to 20 kv. For this wave length range, a commercial sealed-off diffraction type of X-ray tube with beryllium window is used. Auxiliary high vacuum pumping equipment is not required. A simple and inexpensive microradiographic apparatus was assembled in our laboratory two years ago. It consists of a portable cabinet containing a high tension generator converted from a portable diagnostic X-ray unit. A Machlett Type 0-2 diffraction tube with a copper target was mounted externally in horizontal position so as to direct the X-ray beam downward. The focal spot is 1 mm. square in projection at 6 degrees from target face.

The contrast medium. In general, the higher the atomic weight, the better its contrast, and the finer the particle size, the more complete the filling of minute vessels. We have discarded colloidal silver iodide, Urokon, Hypaque, and others, and have found that a mixture of Thorotrast and Micropaque in the following proportions is particularly useful. It consists of Thorotrast, 20 per cent and Micropaque, 25 per cent, made up in

* A very finely ground barium sulfate, supplied by Demancy Co., Ltd., Ware, England.

a 3 per cent A-C-D solution (Abbott Lab., Chicago). The latter was used to minimize intravascular clotting. Methylene blue was added to serve as a visual indicator of the course of the injection. The average particle size of Thorotrast is 0.1 μ, and it easily penetrates the entire capillary network. It is non-irritating, has no effect on blood pressure, and is an excellent medium for demonstration of fine blood vessels. As the particle size of Micropaque varies between 0.1 and 3.0 μ, it provides good filling of larger vessels. With a mixture of Thorotrast and Micropaque, vessels of various sizes are filled and shown in good detail. Carlson and associates[5] suggested the use of Thorotrast and Micropaque in combination for demonstration of the vascular supply of bone.

The photographic emulsion. The emulsion which we have found most satisfactory for microangiographic work is Kodak spectroscopic film 649-GH of 35 mm. width. It has a very low light sensitivity, high contrast, and extreme resolving power (1,000 lines per mm.). The film is developed with Kodak K-19 developer at 68 degrees F. for 5 minutes.

The technique of injection. (i) Kidney. In these studies 15 rats, 4 rabbits, and 20 cats were used. After deep anesthesia with ether or intraperitoneal nembutal, the renal artery was exposed, ligated proximally, and cannulated with 0.045″ I.D. polyethylene tubing. In the rat, instead of renal artery, the abdominal aorta was clamped above, and cannulated below, the level of the renal arteries. A separate ligature was passed around the renal vein but was not tied. The freshly mixed contrast medium was injected with a 20 cc. syringe under moderate hand pressure at the rate of 5-10 cc. per minute (between 60 and 75 mm. of mercury). Each organ was perfused with excess of medium. Immediately upon termination of the injection, the renal vein was tied off and another ligature applied to the artery distal to the cannula. The injected kidney was removed intact and placed in a fixative consisting of eight parts 10 per cent formalin, six parts absolute alcohol saturated with picric acid, one part (1-N) acetic acid, and one part chloroform. The organ was fixed for three days and sectioned at 200 or 250 micra on a freezing microtome. The sections were placed in 50 per cent glycerine to prevent drying before the X-ray exposure was made.

(ii) *Brain.* Prior to injection of the brain, the entire calvarium was removed with rongeurs and the bone edges treated with bone wax. The dura and superior saggital sinus were left intact. The common carotid arteries were exposed in the neck, ligated proximally, and cannulated with polyethylene tubing. Simultaneous bilateral injection of both carotid arteries with contrast medium was carried out with large syringes at a pressure of 70-90 mm. mercury. A total of about 100 cc. was injected in the cat or rabbit. Immediately after injection the jugular veins and distal carotid arteries were clamped and ligated, and the head severed from the body. Any additional oozing of contrast medium was controlled with mass ligatures. The dura was carefully opened and the entire head placed in fixative for several days. The brain was then removed from the skull and allowed to fix for one to two weeks. Brain sections were cut at 200 μ on a freezing microtome and placed in 50 per cent glycerine.

Mounting and radiographing of specimen. In order to obtain maximal definition and minimal blurring of the image, the specimen should be in direct contact with the emulsion. However, in order to protect the emulsion from the wet specimen, which

soaks the emulsion and reduces film sensitivity, a thin sheet of Saran Wrap* was interposed between the film and specimen. It casts no shadow on the film and produces only imperceptible blurring of the image. The mounted specimen and film were then transferred to a 50 x 50 mm. specially made cassette for X-ray exposure. The optimal target film distance is 25 cm. Exposure time varies from 10 to 15 minutes with the thickness of the specimen, at 20 kv. and 15 ma.

Enlargement. Initial enlargement was achieved with a Zeiss photomicroscope for 10 to 60 power magnification. The resolving power was better than 1 μ. Further enlargement was obtained by using a photographic enlarger for additional 4 to 6 power magnification.

RESULTS

Representative microangiographs of the vascular patterns of normal and some pathological kidneys and brains are presented.

(i) *Kidney.* A cross-section through the mid-portion of a normal kidney of cat, rabbit, and rat respectively demonstrates the pattern of renal cortex and medulla. It is of interest to note that the ratio of the thickness of cortex to medulla varies considerably among the three species, the cortex of the rabbit kidney being the thinnest (Fig. 1, parts A, B, C). The orderly arrangement of glomeruli and arterioles in the cortex, and the radial pattern of vasa recta in the medulla of the rat kidney of Figure 1, C, are well demonstrated (Fig. 2). The afferent and efferent arterioles and the arteriolar coils in the glomerular tufts, together with abundant capillary networks, are shown in a cat kidney under higher magnification (Figs. 3, 4).

A microangiograph of vascular injury in an experimental renal abscess, produced by *E. coli* infection, in a rat kidney is shown (Fig. 5). Further enlargement of the same section demonstrates destruction of vessels and loss of glomeruli in and along the marginal zone of the abscess (Fig. 6).

(ii) *Brain.* A coronal section through the rostral diencephalon of a normal cat brain demonstrates a difference in vascular pattern between subcortical nuclear structures (caudate nuclei) and the white matter of the internal capsule (Fig. 7). These structures, as revealed by their patterns in the microangiograph, correspond exactly to their configuration in normal thionin-stained sections. A microangiograph of normal cat neocortex (Fig. 8) demonstrates a striking parallel pattern of arteriolar branching from pial vessels into the gray matter. As the vessels enter the subcortical white matter, they appear to turn and run in a different direction along the course of the nerve fibres.

* Made by Dow Chemical Company. It is a copolymer of polyvinyl chloride and is approximately 0.0005 inches in thickness.

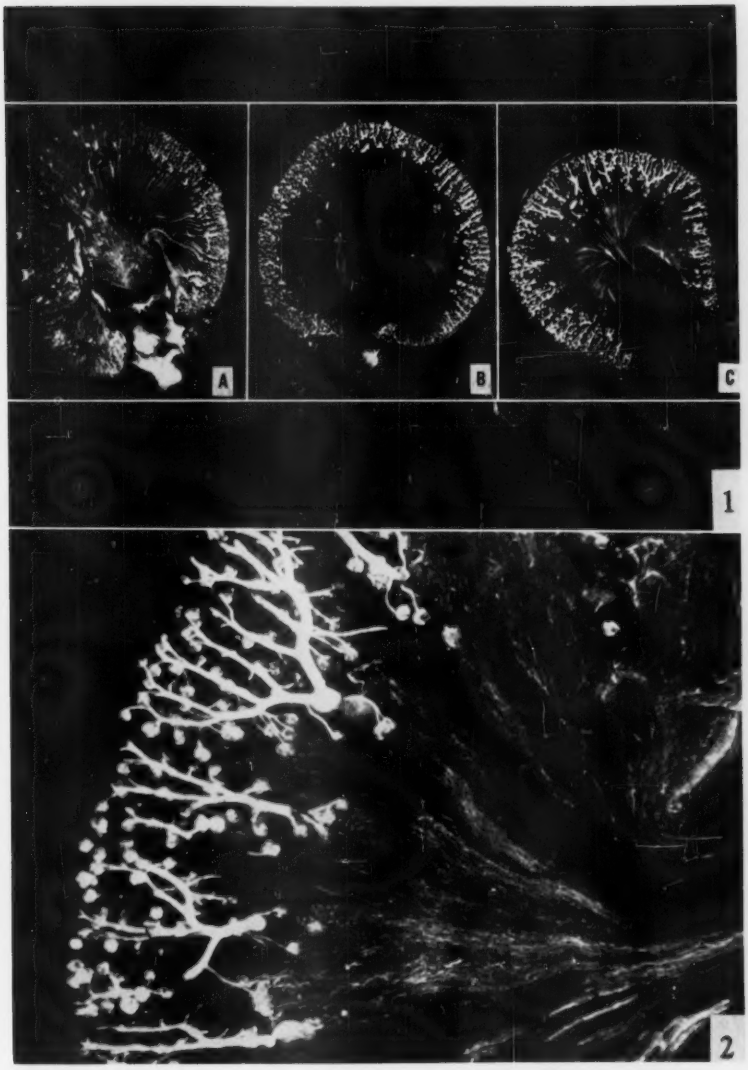


FIG. 1. Microangiograms of normal kidney. (Cross section, 250 μ). A. Cat, x2. B. Rabbit, x2. C. Rat, x5.

FIG. 2. Microangiogram of normal rat kidney from Fig. 1, C. (Section, 250 μ). Detailed vascular patterns in cortex and medulla, x40.

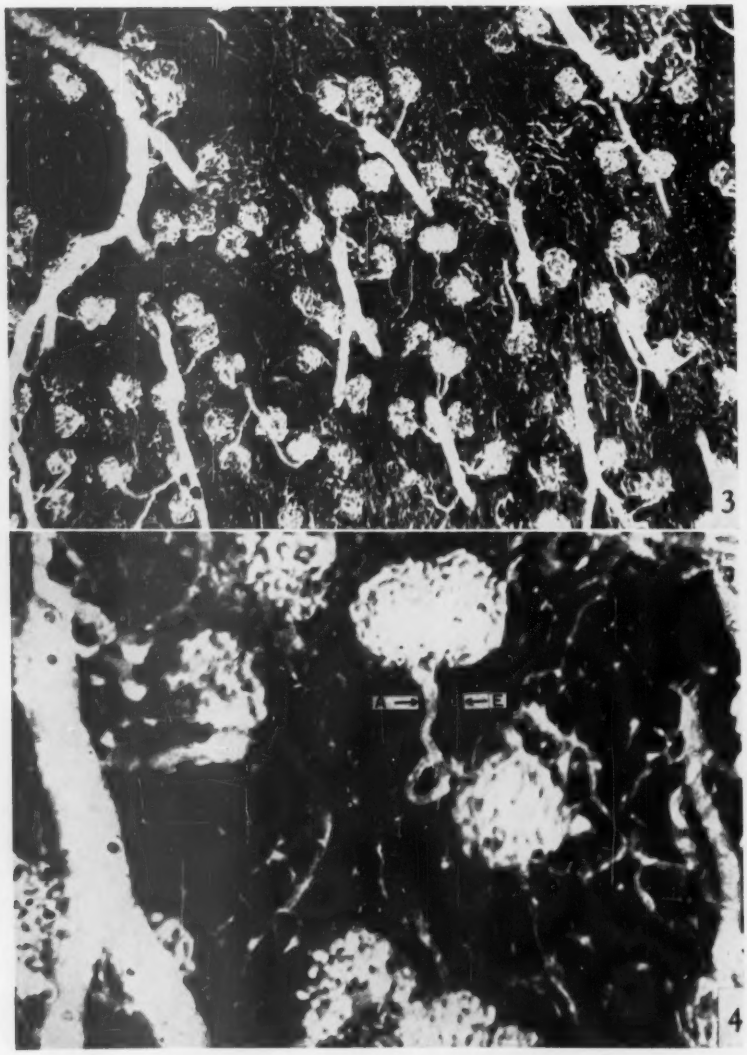


FIG. 3. Microangiogram of normal cat kidney. (Section, 200 μ). Interlobular arteries, afferent and efferent arterioles, glomeruli and capillary networks are demonstrated, x45.
FIG. 4. Same section, x180.

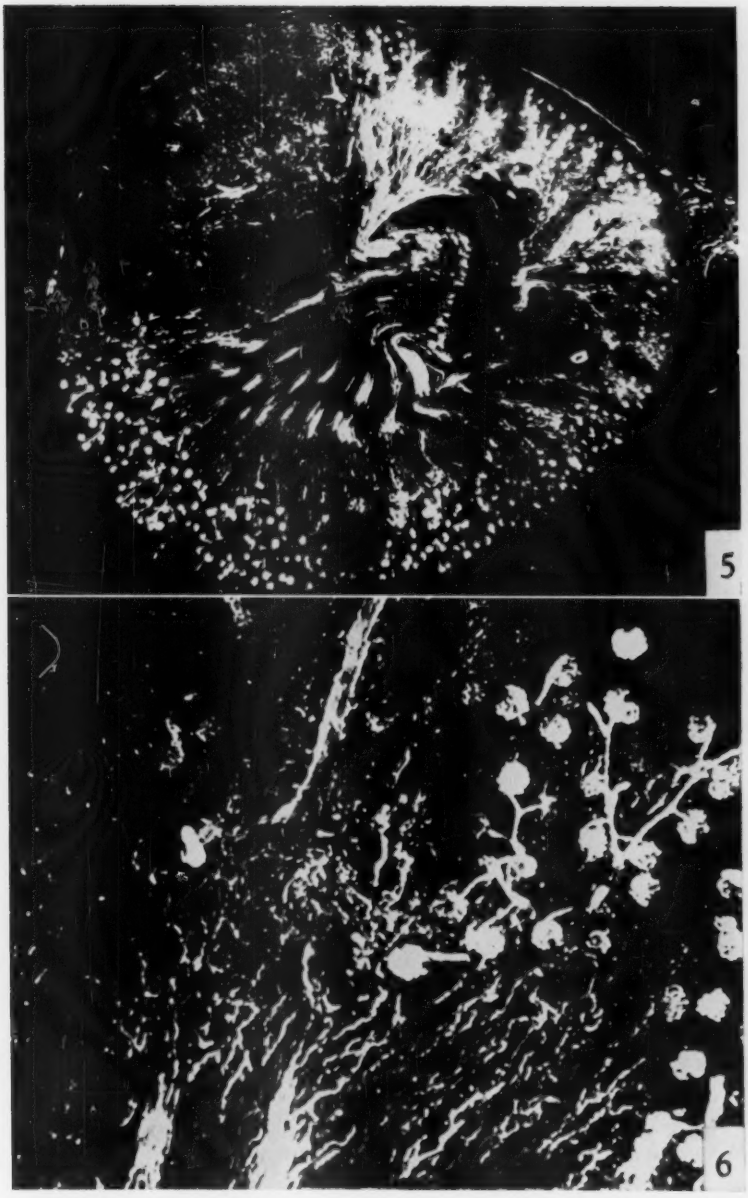


FIG. 5. Microangiogram of infected rat kidney (Section 250 μ). Destruction of glomeruli and vessels in and around a renal abscess, x14.
FIG. 6. Same section, x80.

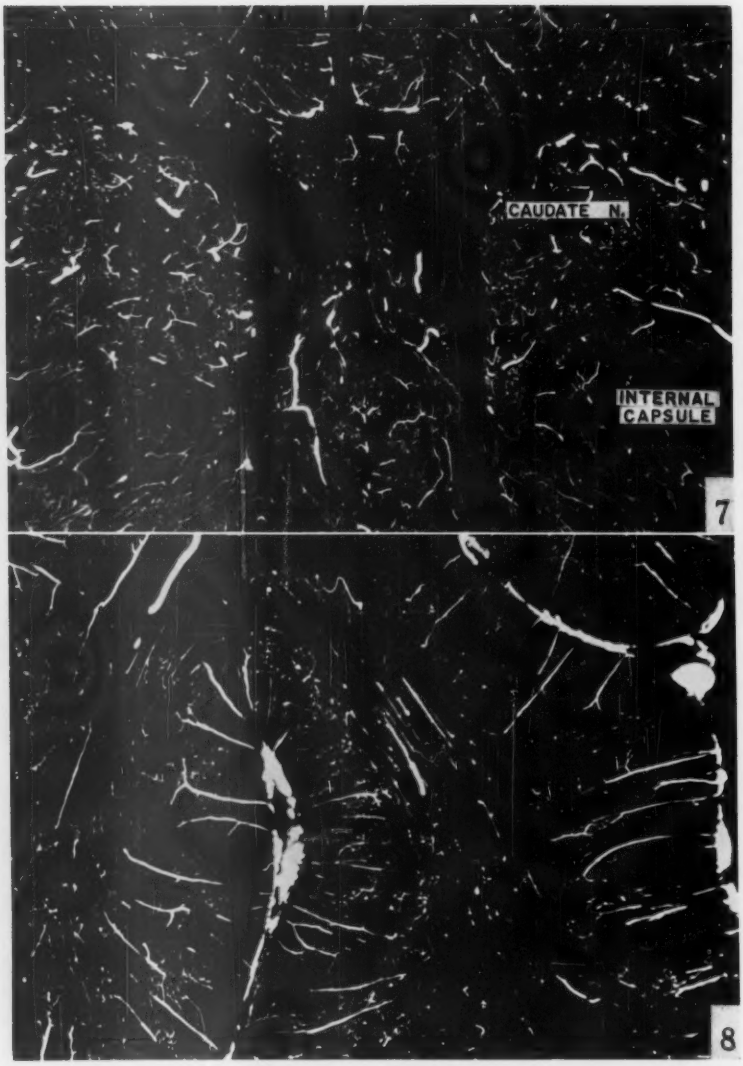
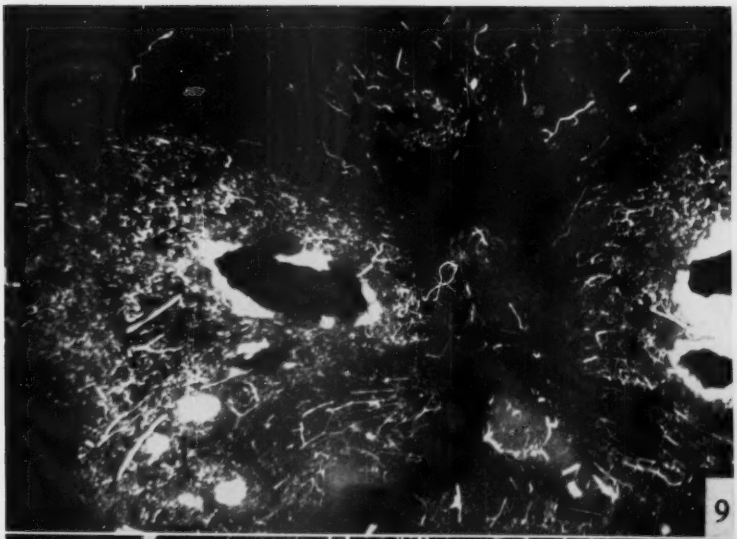


FIG. 7. Microangiogram of normal cat diencephalon, (coronal section, 200 μ). Difference of vascular pattern of subcortical grey and white matter, x14.

FIG. 8. Microangiogram of normal cat cerebral cortex, (Section, 200 μ). Difference of vascular pattern of cortical grey and white matter, x24.



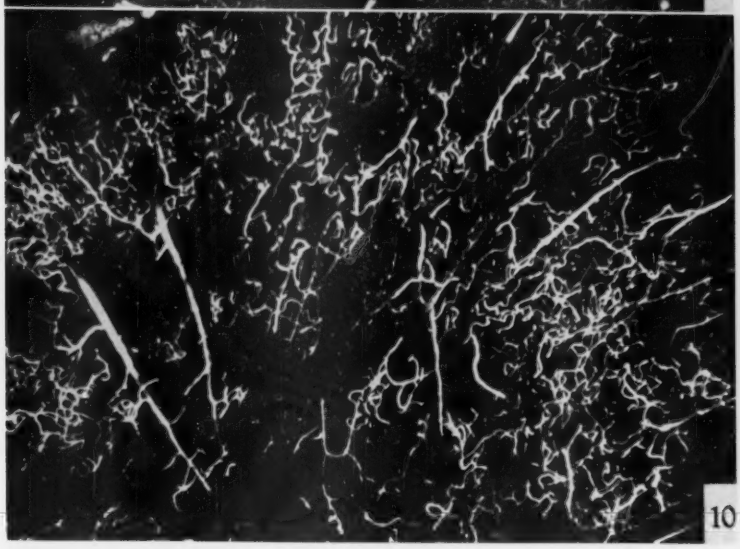


FIG. 9. Microangiogram of irradiated cat mesencephalon (Section, 200 μ). Multiple vascular lesions in pyramids, x16.

FIG. 10. Microangiogram of irradiated cat cerebral cortex. (Section 200 μ). Marked vascular congestion and tortuosity, x45.

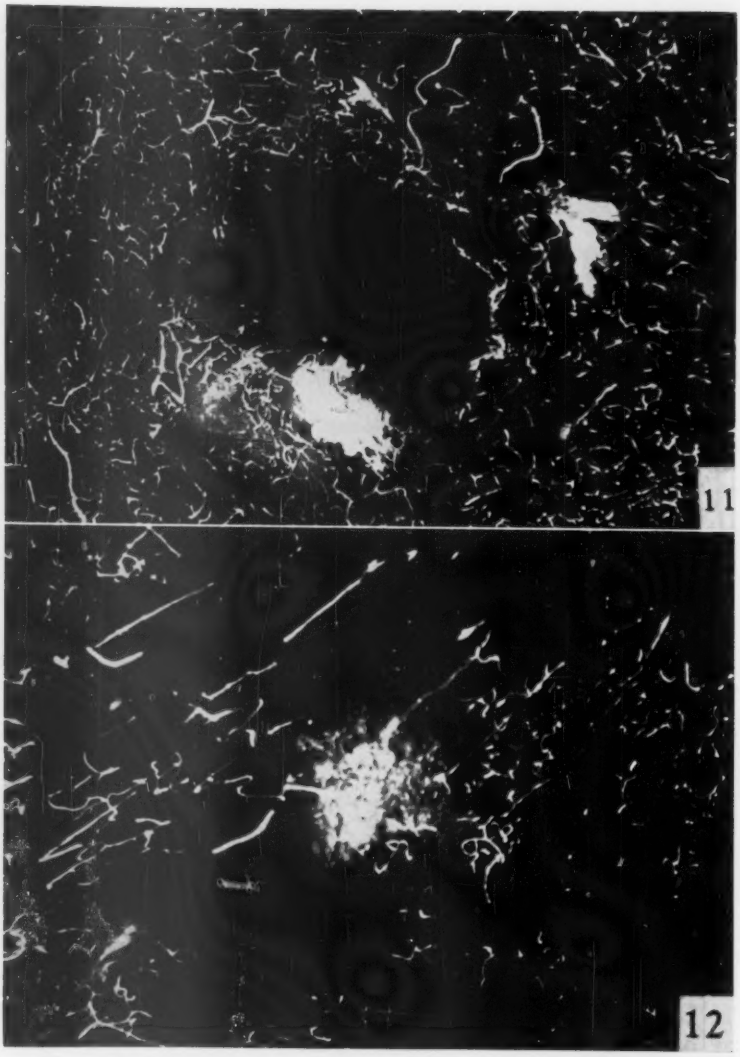


FIG. 11. Microangiogram of irradiated cat thalmus. (Section 200 μ). A necrotic lesion with zonal congestion and extravasation of contrast medium, x45.

FIG. 12. Microangiogram of irradiated cat cerebral cortex. (Section, 200 μ). One of many focal hemorrhagic lesions, x50.

A cross section of an irradiated cat mesencephalon demonstrates marked vascular congestion, extravasation of contrast medium, and necrosis in the ventral quadrants (Fig. 9). This cat received total brain X-irradiation of 2000 r (tissue dose) in a single session using orthovoltage and bilateral opposing ports. A marked spastic quadriplegia developed during the seven-day period prior to sacrifice at one month after exposure. Distortion, tortuosity, and congestion of vessels in the gray matter of the cortex is evident (Fig. 10) as compared with the orderly vascular pattern of normal cortex as seen in Figure 8. A necrotic lesion due to radiation, with zonal congestion and extravasation of contrast medium around a small necrotic cavity, is illustrated (Fig. 11). A punctate collection of contrast medium within a group of disrupted vessels, indicative of a focal hemorrhage, is shown in higher magnification (Fig. 12). This type of lesion is diffusely present in irradiated brain.

DISCUSSION

While excellent techniques for study of the anatomy, physiology, and pathology of large and medium-sized blood vessels have long been available, there is an apparent lack of suitable techniques for study of morphology and function of minute vessels. The microangiographic technique seems to fill this need, as it has the advantage of being able to demonstrate vascular patterns of minute vessels within an intact specimen in reasonably good focal depth. A paired picture can be taken for viewing under a stereoscopic microscope.

Since the resolution of present microangiography is in the order of 1 μ or less, a major step toward further technical improvement would lie in the direction of increasing the contrast of the smaller vessels in relation to the surrounding tissues. This can be done along the following lines:

(a) Improvement of contrast media—a search for more opaque, less toxic and non-radioactive agents.

(b) Prevention of vascular spasm, or induction of vasodilation before and during injection of the medium by using various pharmacological agents.

(c) Reduction of leakage of contrast medium during and following injection, especially when injecting brain vessels. During the process of decapitation and removal of the brain from the skull, considerable amounts of contrast medium may leak away.

The long X-ray exposure time is a limiting factor if microangiographic technique is applied to the study of living tissues. As only the vascular shadow is of interest, the details of surrounding tissue can be ignored. With

a highly radiopaque contrast medium, a higher kilovoltage technique can be used and the exposure time cut down considerably. Experiments along this line are in progress.

The production and demonstration of vascular lesions in brain following X-irradaition is of considerable clinical interest and significance. Experiments designed to test the normal tissue tolerance dose range of kidney, brain, and intestinal tract with this technique are in progress. Accurate information in this regard is urgently needed in radiotherapy.

The microangiographic technique is not as difficult and complicated as it was thought to be and the expenditure for setup of a unit is not beyond the possibilities of any average scientific laboratory. With continued improvement of equipment and technique, microangiography can be expected to be widely used for investigation in the fields of experimental surgery, pathology, and physiology where information concerning detailed morphology in microcirculation is sought.

SUMMARY

(i) A brief survey of the history of microradiography and microangiography is made.

(ii) A simple microangiographic technique utilizing relatively inexpensive apparatus, as used in our laboratory, is described.

(iii) The normal vascular patterns of kidney and brain are described and illustrated. Some vascular lesions of rat kidney and cat brain following bacterial infection or local X-irradiation are presented.

(iv) The potentialities and limitations of the microangiographic technique are briefly discussed.

ACKNOWLEDGMENT

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A NEW PROPERTY OF "COMPLETE" ANTIGEN O OF GRAM-NEGATIVE BACTERIA: ITS SPECIFIC PROTECTIVE EFFECT ON THE SENSITIVITY OF *STAPHYLOCOCCUS AUREUS* TO PENICILLIN†

Zablocki *et al.*[5,8,9,10] have established that antigen O preparations isolated from bacilli of the family Enterobacteriaceae have a protective effect on the sensitivity to penicillin of gram positive bacteria (*Staphylococcus aureus, Staphylococcus albus, Streptococcus pyogenes, Corynebacterium diphtheriae, Sarcina lutea,* and *Diplococcus pneumoniae*). The results of the considerably expanded investigation form the basis of the present communication.

MATERIALS AND METHODS

The antigen O was isolated from two smooth strains (HZ,U) of *Escherichia coli* employing the methods of Boivin,[2] Zablocki,[11] Morgan,[4] and Westphal.[6] A commercial preparation of *E. coli* (Difco, bacto-lipopolysaccharide) was also used.

The method of Zablocki for the isolation of the O antigen is as follows. A heavy bacterial suspension ($5x10^{10}$ cells) is washed three times in distilled water and then suspended in water to the original volume. This suspension is frozen at —20° C. for 16 hours and then thawed at 37° C. for 8 hours. This procedure is repeated 15-20 times. The resulting autolysate is then centrifuged (8,000 rpm.) for one hour and the sediment discarded. The supernatant is filtered through a bacterial filter (Chamberland L_1 followed by L_3). To the opalescent filtrate after dialysis approximately 10 volumes of 95 per cent alcohol and 5 ml. of a saturated solution of NaCl are added. The resulting sediment (denatured protein and O antigen) is dried in vacuum over P_2O_5. The dried material is ground in a mortar with the gradual addition of distilled water to 100 ml. (denatured protein not going into solution). After centrifugation at 8,000 rpm. for 30 minutes, the sediment is discarded and the supernatant containing the O antigen is lyophilized dry.

Staphylococcus aureus strain NHL is highly sensitive to the action of penicillin (inhibitory dose = 0.048 units/ml.).

Antibiotics used in the experiments were penicillin G, bacitracin, vancomycin, staphcillin, and kanomycin.

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** Associate in Research in Microbiology.

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Protective effect technique. To each of 15 Wassermann tubes 0.5 ml. of a suspension of 200,000 cells/ml. of *Staphylococcus aureus* NHL was added. Then, 0.5 ml. of the proper sterile O antigen dilution was added, and the resulting mixture incubated for 1 hour at 37° C. After incubation, 1.0 ml. portions of pencillin G, serially diluted (200 units to 0.01 units/ml) in Difco nutrient broth, were added to tubes 1-14. Tube #15 was a control lacking penicillin. A second control consisted of a row of

TABLE 1. PROTECTIVE EFFECT OF ANTIGEN O ON *Staphylococcus aureus* (NHL) AGAINST PENICILLIN G

Tube number	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
*Penicillin G units/ml.**	*100*	*50*	*25*	*12.5*	*6.25*	*3.1*	*1.5*	*0.7*	*.39*	*.19*	*.09*	*.04*	*.02*	*.01*	*control*
Zablocki antigen															
prep. #1	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
prep. #2	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Boivin antigen															
prep. #1	—	—	—	—	—	—	—	—	—	—	—	±	+	+	+
prep. #2	—	—	—	—	—	—	—	—	—	—	—	±	+	+	+
Morgan antigen															
prep. #1	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+
prep. #2	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+
Westphal antigen															
prep. #1	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
prep. #2	—	—	—	—	—	—	—	—	—	—	—	+	+	+	+
Difco antigen	—	—	—	—	—	—	—	—	—	—	—	±	+	+	+
Control	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+

* Final concentration.
— No growth.
+ Abundant growth after 24 hrs. at 37° C.
± Weak to no growth.
Final concentration of antigens are 0.1 per cent.

15 tubes, each containing 0.5 ml. of saline in place of the antigen. The results of bacterial growth were read after 24 hours' incubation at 37° C. All procedures were performed under sterile conditions.

When the solution of antigen O was highly opalescent, determination of growth was carried out in the following medium: nutrient broth, 1 per cent dextrose, and brom cresol purple as an indicator. A change in indicator color from purple to yellow denoted growth.

RESULTS

The results of the comparative investigation of the protective activity of the various preparations of the O antigens are presented in Table 1.

From the data in Table 1 the following conclusions can be made:

1. Both of the preparations after Zablocki are highly effective in protecting *S. aureus* NHL against the action of penicillin. It can be seen that an inhibitory dose of 100 units per ml. was required as compared with 0.04 units per ml. for the control without antigen O.

2. Of the two preparations of antigen O, after Morgan, only the first proved effective in the protection of the bacterium against penicillin; the activity, however, was much below that of Zablocki's antigen.

TABLE 2. MINIMUM PROTECTIVE DOSE ZABLOCKI ANTIGEN O ON *Staphylococcus aureus* AGAINST PENICILLIN

Conc. antigen (μg/ml.)	*Inhibitory dose Penicillin**	*Inhibitory dose Control dose*
No antigen	0.0976	1
15.6	0.0976	1
31.25	0.0976	1
62.5	0.195	2
125.0	1.56	16
250.0	6.25	64
375.0	25.0	256
500.0	25.0	256
625.0	50.0	512
750.0	50.0	512
1000.0	100.0	1024

* Penicillin—units/ml.
** Control dose.

3. The preparations of the O antigens after Boivin, Westphal, and Difco showed no protective effect on *S. aureus* NHL against penicillin.

The minimum protective dose of the antigen O after Zablocki is presented in Table 2.

The protective activity of the O antigen appears only against penicillin, and is therefore highly specific (Table 3). It is interesting to note that antigen O shows no protective activity against "Staphcillin," a synthetic penicillin which has been shown to inhibit the growth of staphylococci which are resistant to the action of penicillin.

Other antibiotics (streptomycin, erythromycin, aureomycin, terramycin, and chloromycetin) were also investigated by Zablocki *et al.*[5] and were shown to be unaffected.

The mechanism of the protective activity of the O antigen. In a previous paper Zablocki and Rolicka[8] have shown that there is no direct effect of antigen O preparations from gram negative bacteria on penicillin itself. It is thus postulated that the mechanism for the protective activity of the O antigen against the action of penicillin on *Staphylococcus aureus* is due

TABLE 3. SPECIFIC PROTECTIVE EFFECT OF ANTIGEN O

Tube number	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Antibiot. conc. (μgm/ml.)	*625*	*312*	*156*	*78.1*	*39*	*19*	*9.7*	*4.8*	*2.4*	*1.2*	*0.6*	*0.3*	*0.1*	*0.07*	*control*
Antigen + Bacitracin*	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Control**	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+
Antigen + Vancomycin	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+
Control**	—	—	—	—	—	—	—	—	—	—	—	—	±	+	+
Antigen + Staphcillin	—	—	—	—	—	—	—	—	—	—	+	+	+	+	+
Control**	—	—	—	—	—	—	—	—	—	—	±	+	+	+	+
Antigen + Kanomycin	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+
Control**	—	—	—	—	—	—	—	—	—	—	—	—	—	±	+
Penicillin conc. units/ml.	*100*	*50*	*25*	*12.5*	*6.2*	*3.1*	*1.5*	*0.7*	*0.3*	*0.1*	*.09*	*.04*	*.02*	*.01*	*control*
Antigen	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Control**	—	—	—	—	—	—	—	—	—	—	—	—	+	+	+

* Units /ml.

** Appropriate control without antigen.

to the "coating" of the surface of the bacterial cell with molecules of the antigen. This coating presumably causes an inhibition in the transport of the antibiotic across the surface of the sensitive cell. It is also thought that the surface of the gram positive cell has some chemical affinity for the O antigen, and is able to absorb this from solution. In support of the above postulated mechanism are the following experiments and results:

1. After incubation of staphylococci for one hour at 37° C. with antigen O after Zablocki, the cells were centrifuged for two hours and then washed

with 0.85 percent NaCl. The cells were found to preserve their resistance to penicillin, as is shown in Table 4.

2. An estimation of the total carbohydrate concentration (Anthrone Method) in the supernatants, after incubation at 37° C. and centrifugation of the staphylococcus cells mixed with antigen O (after Zablocki), showed that a significant amount of the antigen was removed from solution (Table 5). Since there is a small quantity of carbohydrate in the control tube (#4), one must subtract this from the amount of total carbohydrate in the test tube (#1).

TABLE 4. EFFECT OF WASHING AND CENTRIFUGATION UPON RESISTANCE OF *Staphylococcus aureus* "COATED" WITH ANTIGEN O OF ZABLOCKI

Tube number	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
*Penicillin G units/ml.**	*100*	*50*	*25*	*12.5*	*6.2*	*3.1*	*1.5*	*0.7*	*0.3*	*0.1*	*.09*	*.04*	*.02*	*.01*	*control*
Staph. + antig. (centr. & wash)	ng	ng	G	G	G	G	G	G	G	G	G	G	G	G	G
Staph. + saline (centr. & wash)	ng	ng	ng	ng	ng	ng	ng	ng	ng	ng	ng	ng	G	G	G

* Final concentration.
ng—No visible growth.
G—Visible growth.

DISCUSSION

The results of this series of experiments raise the question of the existing differences among the various preparations of the O antigens. The chemical structure of the native antigen O in the bacterial cell wall is as yet not known for any species. By diverse chemical methods of extraction and purification, many investigators have been able to obtain O antigens or lipopolysaccharides which appeared to be different in their chemical properties, but preserved their serological (bound with the polysaccharide moiety) and toxic activities. Goebel *et al.*[1] have found that the toxin component T is bound with the protein or polysaccharide, depending upon the method of hydrolysis. Westphal[7] concludes that the toxic and pyrogenic activities are caused by lipid A.

From the five different preparations of the O antigen, as mentioned above, only the ones isolated after Zablocki or Morgan protect antibiotic-sensitive gram positive bacteria against penicillin, although not against a variety of other antibiotics.

It is hypothesized that the natural resistance of the gram negative bacilli against the action of penicillin is probably due to the chemical structures of their surfaces, which contain, as we know, the antigen O. Thus, the transfer of this antigen O from solution to the surface of the gram positive bacteria makes the latter also resistant to the action of the penicillin.

We propose to differentiate two types of antigen O: (i) "complete" antigen O of Zablocki and Morgan which, upon being absorbed by the

TABLE 5. TOTAL CARBOHYDRATE IN SUPERNATANTS

Tube number	*1*	*2*	*3*	*4*
Zablocki antigen (0.2 per cent)	5 ml.	5 ml.	5 ml.	—
Staph. suspen. 5.0×10^7	5 ml.	—	—	5 ml.
E. coli suspen. 5.0×10^7	—	5 ml.	—	—
Control—saline (0.85 per cent)	—	—	5 ml.	5 ml.
Total (CH_2O) x (μg./ml.)	125	165	160	10
*Total (CH_2O) x (μg./ml.)	115	0	160	—

* Corrected = Tube 1 minus Tube 4.

staphylococcus cells normally sensitive to penicillin, are able to protect the cells from penicillin action; and (ii) "incomplete" antigen O of Boivin, Westphal and Difco Labs, which have lost their activity for penicillin protection during the process of chemical extraction and purification. For the demonstration of this quality of "completeness" of the antigen O preparation the method that has been described in this paper may be useful.

The surface of the typical gram positive cell is quite different, physicochemically, from that of the gram negative cell (Dubos[8]). On the basis of our investigations we can say that the surface of the gram positive bacteria shows a definite chemical affinity for the components contained in the "complete" O antigen. This affinity is different for different species of the gram positive bacteria (Zablocki *et al.*[5]).

It can thus be concluded that the antibiotic protective effect of the "complete" O antigen is due to the following:

1. The completeness of the O antigen, and

2. The degree of chemical affinity of the surface of gram positive bacteria for the O antigen.

SUMMARY

From the data that have been presented in this paper, as well as in previous reports,[5, 8, 9, 10] the following can be concluded:

1. One must distinguish two types of O antigen: a) "complete" antigen O, which seems to be chemically near to that of the native antigen in the cell wall and has the activity of protecting the gram positive bacteria against the action of penicillin; and b) "incomplete" antigen O, which, during the process of chemical extraction and purification, has lost the ability to protect the cells against penicillin.

2. From the five investigated preparations of antigen O only those isolated by the methods of Zablocki and of Morgan have preserved their protective activity, and therefore can be referred to as "complete" antigen.

3. The protective activity of the "complete" antigen O is highly specific against penicillin but not against the other antibiotics examined.

4. The mechanism of the protective activity of the O antigen depends upon two factors: upon the completeness of the antigen and upon the absorbing ability (chemical affinity) of the surface of the penicillin-sensitive gram positive bacteria for the "complete" O antigen.

5. The natural resistance of the gram negative bacilli against penicillin is probably bound with the chemical structure of their surface which contains the complete O antigen.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. Henry P. Treffers for generously making his laboratory facilities available to us and offering much advice.

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Mother and Daughter with Bilateral Congenital Amastia.

By H. Goldenring and E. S. Crelin, Department of Anatomy, Yale University School of Medicine.

Only three publications were found in which complete bilateral congenital absence of the mammary glands was reported to have occurred in otherwise normal individuals. Wylie[3] described a 21-year-old affected mother of an unaffected son. Williams[2] published additional information, supplied by Wylie, concerning this woman. Fraser[1] described three generations of a family in which bilateral amastia showed a dominant inheritance. The male was the affected individual in the first generation. Three of his four daughters were affected and his two sons were unaffected. One of these affected females had three affected daughters and two unaffected sons. The father of these latter children was unaffected. A three generation study of his family, similar to that made of his affected wife's family, revealed no occurrence of amastia.

The individuals with amastia of the present study are a 25-year-old woman and her 3 ½-month-old daughter (Figs. 1 and 2). Physical examination of the mother revealed, in addition to the apparent amastia, sparse axillary and pubic hair, a high arched palate, and depressed nasal bridge suggesting saddle nose. The teeth are apparently within normal limits with reference to number and configuration. The finger nails, although traumatized by nail biting, show no dystrophic changes, nor do the toe nails. The bony thorax, pectoralis major muscles, and all other findings were within normal limits. Biopsy in the axillary area revealed some hairs, numerous active eccrine sweat glands but no apocrine glands whatever.

The infant's hair and nails appeared normal although absolute evaluation at age 3 ½ months is not possible. The child did show marked hypertelorism, high arched palate, and saddle nose as well as the absence of areolae and nipples. The areas where, normally, the breasts would be on the daughter have the same surface configurations of the breast areas on an unaffected child of a comparable sex, age, and body build. However, the breast areas on the mother are similar to those on a male of a comparable age and body build because each pectoralis major muscle produces a visible elevation of the skin overlying it and there is no excessive accumulation of superficial fat. The mother also has a 3-year-old unaffected daughter. During her two pregnancies no change occurred in the subcutaneous tissue overlying the pectoralis major muscles which indicates a complete absence of mammary gland parenchyma.

The mother has American Indian, French Canadian, Irish, and Scotch ancestry. The 26-year-old father of the affected child is unaffected and has Scotch ancestry. The history of the family of both the mother and the father back to the great grandparents of the infant, data supplied by the

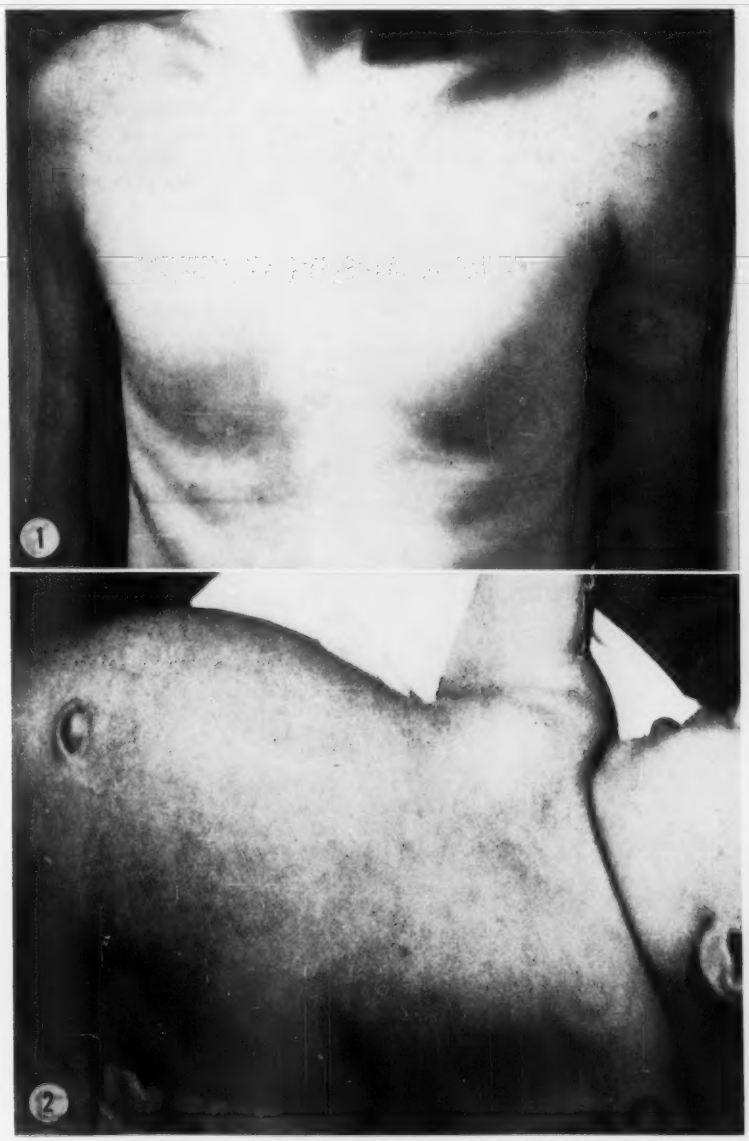

FIG. 1. Anterior view of the thorax of a 25-year-old female with bilateral amastia.
FIG.. 2. Anterior view of the thorax and abdomen of a 3½-month-old female with bilateral amastia. The child's mother is shown in Figure 1.

mother, did not reveal an occurrence of amastia. Since the history was largely second-hand information and incomplete, it is not possible to ascertain whether the amastia was transmitted as a hereditary trait to the affected mother or whether she is a mutant.

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BOOK REVIEWS

CELL PHYSIOLOGY OF NEOPLASIA. A collection of papers presented at the 14th Annual Symposium on Fundamental Cancer Research at the University of Texas, M.D. Anderson Hospital and Tumor Institute. Austin, University of Texas Press, 1960. 653 pp. $10.50.

This book is a collection of papers presented at the 14th Annual Symposium on Fundamental Cancer Research at the University of Texas M.D. Anderson Hospital and Tumor Institute. It is divided into four sections: the nucleus, the cytoplasm, the nucleic acids, and cell growth and development. It brings together a very wide group of investigators working in entirely different fields, principally interested in cellular metabolism, although usually only incidentally in tumor metabolism. It is obvious that no real qualitative differences have been found between tumor and normal cells and thus the emphasis on intermediary pathways and developmental cell biology, without specific reference to cancer, appears to be well justified.

The chapter on the nucleus discusses chromosomal physiology, studies on the nucleolus and structural organization of the nuclear envelope. Perhaps in the future there will be investigators able to combine this section with the section on nucleic acids. The latter chapters describe studies on the structure, composition, and metabolism of deoxyribonucleic acid and ribonucleic acid. Although current symposia usually include workers from both the structural and biochemical fields in an attempt to get cross-fertilization and greater understanding, it is rare to find someone who adequately understands and interrelates these two approaches. There is a particularly good chapter concerning the variation in cellular nucleic acid produced by viruses. The introduction of a known carcinogenic agent such as a mammalian virus into the cell and the investigation of morphological and chemical events following infection will probably be as profitable in the future as were the earlier studies with phage bacteria. The electron micrographs of Dmochowski on polyoma virus in the chapter on the cytoplasm are outstanding. The last section on cell growth has little relation to tumor, but presents very interesting studies by workers in cell differentiation and embryology.

Perhaps the most interesting part of the book is the Bertner Foundation lecture by E. V. Cowdry in which he discusses the lives of cancer cells. The effect of viruses on cells, and cellular susceptibility to chemical carcinogens may be closely related to inherent cell types, i.e. intermitotics, reverting post mitotics, or fixed post mitotics. Doctor Cowdry has been involved in the field of cancer research for a period of over 40 years and has the perspective to combine some of the newer knowledge with some of the very important older facts about cellular physiology and morphology. Unfortunately, much of this latter work has been forgotten by a large number of workers in cellular biology today. Although he is not particularly concerned with purine or pyrimidine base ratios, or the amino acid sequence of

abnormal tumor proteins, his basic philosophy and ideas concerning cellular capabilities are most informative. The summary chapter by M. J. Kopac summarizes much of the current pertinent knowledge on cellular differentiation, mechanisms of information transfer and DNA-RNA interrelationships. The book is well written, well illustrated, and should be well received.

DONALD W. KING

PROTEIN AND AMINO ACID REQUIREMENTS IN EARLY LIFE. By L. Emmett Holt, Jr., Paul Gyorgy, Edward L. Pratt, Selma E. Snyderman, and William M. Wallace. New York University Press, 1960. 63 pp. $1.

This small volume presents a well-written, critical, and (considering its size) reasonably comprehensive review of the current knowledge of protein nutrition in early childhood. The authors are pediatricians who have distinguished themselves by investigative work in the field. Data from the literature as well as previously unpublished material are evaluated with regard to applicability to clinical problems. A discussion of the epidemiology of protein malnutrition is included. The book is well worth the brief time required to read it, and the extensive bibliography should enable the reader to proceed directly to those aspects of the field in which he has a particular interest.

IRA K. BRANDT

INTRODUCTION TO A SUBMOLECULAR BIOLOGY. By Albert Szent-Györgyi. New York, Academic Press, 1960. 135 pp. $5.00.

Dr. Szent-Györgyi's latest work, a sequel to his BIOENERGETICS (Academic Press, New York, 1957), reflects an evolution in his thought and research. He urges a renascence in the life sciences, including collaboration of biologists and physicists in the study of the electronic interactions of biological molecules. Although the biosynthesis of ATP has been reasonably well documented and most, if not all, hormones and vitamins have been isolated and characterized, "the really intriguing problems . . . (are) not what these substances *are*, but what they *do*, how they act on the molecular level, and how they produce their actions." Since man is presently making little progress in solving these problems, "something very important, a whole dimension, might be missing from our present thinking without which (they) cannot be approached." This dimension is the submolecular (electronic), the nature of which endows each atom of a molecule with a "personality" not indicated by the classical formula. Although Szent-Györgyi can admittedly only ask the proper questions (and by so doing show that an intelligent teleological approach is a necessary part of a scientist's intellectual armamentarium), he hopes that "the elucidation of these interrelations may eventually lend to our thinking the plasticity which may be necessary to approach life and the meaning of that unique system called the 'cell'."

After a most refreshing discussion of the generation of energy through ATP and the oxidative cycle, the author outlines the methods of quantitating electron donor or electron acceptor molecules. He briefly considers the biological role of electromagnetic coupling and semiconduction in electronic mobility. However, he deals more extensively with charge transfer which "may be one of the most important, frequent, and fundamental biological reactions." A chapter is devoted to methods of detection of charge transfer, differentiation of weak and strong charge transfer, and the possibility of understanding biological phenomena through this mechanism. Three pertinent examples of charge transfer are discussed: quinone-hydroquinone, serotonin-flavinemononucleotide, and ketosteroid- (cortisone-) iodine. This application of his theory by means of simple, dramatic experiments is the highlight of the book. Further speculations on the biological importance of charge transfer comprise the final five chapters.

Szent-Györgyi unfortunately has simplified critical elements of his theory to the point of near meaninglessness. A more detailed discussion of orbital energies, including a definition of the "k"-value and a qualitative derivation should have been included. The description of electron spin resonance, the most important tool in detection of free radicals and charge transfer complexes, is also too brief and vague. The experimental data on the ketosteroid-iodine charge transfer complexes would be more meaningful had Mullikin's "outer" and "inner" complexes been defined.

Despite these criticisms this book is certain to be a revelation to researchers in the biological, biochemical, biophysical, and quantum mechanical sciences. For the biologist and biochemist, a new approach to the understanding of life processes through intimate electronic processes, rather than through exchange of atoms, is outlined. To the physicist, a new field of research, promising even greater intellectual challenges and rewards, is presented.

P. V. T.

RECENT PROGRESS IN HORMONE RESEARCH. (Proceedings of the Laurentian Hormone Conference 1959). Vol. 16, Gregory Pincus, Ed. New York, Academic Press, 1960. viii, 608 pp. $14.00.

This book contains the proceedings of the 1959 Laurentian Hormone Conference and, like its predecessors, is a worth-while effort.

Four sections comprise the book: mechanism of hormone action, peptide hormones, thyroid hormones, and hormones and metabolism. Topics in all four sections are well covered by all the participants with respect to a detailing of the current information on the subject. Particularly noteworthy are the conflicting theories of Tallalay and Villie on the physiological functions of steroid hormones with respect to the enzymatic transfer of hydrogen: the accumulating evidence tends to disprove Tallalay's theory of a single 17 β-hydroxysteroid dehydrogenase in the placenta which catalyzes estrogen-dependent transhydrogenations.

For the reader who is interested in hormone research, the minutes of these proceedings are a recommended purchase.

J. R. COOPER

THE INTELLIGENT MAN'S GUIDE TO SCIENCE. By Isaac Asimov. New York, Basic Books, Inc., 1960. 2 vols. 767 pp. $15.00.

The phrase "on historical principles" might well have been added to the titles of these volumes, for most sections are presented historically—much too historically in some cases. For instance, the section on mechanisms of evolution proceeds from Cuvier and Lamarck to Darwin, Wallace, and Huxley, and then concludes with the Scopes trial. If anyone wishes to know current concepts of evolutionary mechanisms, he won't find them here.

Isaac Asimov is Associate Professor of Biochemistry at Boston University and has produced 767 pages of well-written text plus nearly a hundred pages of bibliography, credits for illustrations, and an index. The first volume discusses the physical and the second, the biological sciences. Within each volume the organization is not patterned on standard basic texts but is enriched with imagination. And Asimov does write well.

However, he has attempted to cover more material than 767 pages can contain. The publisher describes the book as "written for the layman and for the specialist as well." Only occasionally will the specialist experience anything other than frustration in using these volumes. The work will be of interest to those who know little or nothing of science, but they will not always be introduced to current science.

FREDERICK G. KILGOUR

BASIC THEORY OF PSYCHOANALYSIS. By Robert Waelder. International University Press, 1960. 273 pp. $5.00.

This book is broad in scope and an important contribution to the growing efforts to clarify the scientific basis of psychoanalysis. It attempts to encompass the totality of scientific psychoanalysis and present it to a heterogeneous group of readers. Dr. Waelder lists "three goals: to combat widespread misunderstandings . . .; to see psychoanalysis in its context in the history of our civilization; and to help discover the most promising avenues of advancement." This approach makes the book especially useful for the young psychiatric resident very often viewing psychoanalysis only in its more restricted technical aspects. It should help him see the unfolding of the development of psychoanalysis as a young science and how it stands in relation to the more firmly established physical sciences. Dr. Waelder writes from a rich background of experience in the mainstream of psychoanalysis. He represents by his very example an effective reply to critics of the field who maintain that it is static and too reliant on early formulations developed by Freud.

If there is a difficulty in the book, it is in the very vastness of the project undertaken. The scientific underpinnings of psychoanalysis are developed, the basic theoretical concepts of psychoanalytic psychology are described in historical sequence, clinical syndromes are explained, theoretical and practical aspects of treatment are outlined. All this is done with skill and with a refreshing sense of timing and wit so rare in serious medical writings.

Though this book will find a varied audience among the intellectual non-medical public, it is this reviewer's opinion that it will be most meaningful to the young psychiatrist or physicians seriously interested in psychiatry. Dr. Waelder writes gracefully and makes his points easily but these points are often far-reaching and require careful consideration by an alert reader.

EDWIN C. WOOD

Articles are accepted for publication in *The Yale Journal of Biology and Medicine* on the basis of merit and suitability. The privilege of submitting articles is not limited to those associated with Yale University and contributions from others are cordially invited.

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